Seventy Generations of Selection for Oil and Protein in Maize

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Preface

The primary objective of the Crop Science Society of America is to promote the public welfare by advancing research, extension, and teaching of all basic and applied phases of the crop sciences. With the publication of this book, the Society is taking another step toward fulfilling this obligation by making available in one compilation the results of 70 generations of selection for oil and protein in maize. This ongoing project is unique in its longevity, and publication of these results is timely because of increased awareness of the importance of quality in food and feed crops. Hopefully, the results reported here will provide both useful information and inspiration to plant breeders and geneticists around the world.

We are indebted to the University of Illinois and to all of the scientists who have worked on the project over the years for carrying this work through 70 generations of breeding. We are particularly indebted to Dr. John W. Dudley, the Editor and a contributing author, his coauthors, and Drs. S. A. Eberhart and R. H. Moll whose objective reviews made important contributions to this book.

February 1974

R. R. Davis, President
Crop Science Society of America

Foreword

In 1896 at the Illinois Agricultural Experiment Station, C. G. Hopkins, a chemist by training, began to select for grain oil and protein percentage in Burr's White, an open-pollinated variety of corn. The experiment has continued at Illinois, with some modifications, through the perseverence of L. H. Smith (1900 to 1921), C. M. Woodworth (1921 to 1951), and E. R. Leng (1951 to 1965) until J. W. Dudley, R. J. Lambert, and D. E. Alexander assumed responsibility in 1965. The long time span of this experiment indicates an early and continued interest in the feeding quality of corn grain.

The experiment was conceived and initiated before the rediscovery of Gregor Mendel's paper. There can be little doubt that Darwinism affected the initiation of the experiment, although Hopkins, as far as can be ascertained, did not mention the theory in his publications. He did cite the success of selection in animals and in increasing sugar concentration in the beet as evidence that selection should be effective in modifying chemical composition of the corn kernel. However, the magnitude of the changes encountered during the experiment vastly exceed anything Hopkins must have envisioned.

The extent of these changes over 70 generations and the interpretation of their meaning are documented in the last chapter in this volume. However, a full appreciation of this classical experiment requires more than a brief look at the final results. Thus, included in this volume are key papers showing the evolution of the experiment and the philosophy of some of the researchers associated with it. The detailed data from each generation provided in the separate segments of this report will, hopefully, encourage others to reevaluate the experiment and perhaps provide additional interpretations.

In many of these papers, predictions of future progress in the experiment have been made. It is remarkable that most of the predictions were later refuted by further advances in the direction of selection. However, this experience does not deter us from predicting, as this experiment continues, that the 140th generation will provide information as unexpected to us as that of the 70th generation would have been to Hopkins in 1896.

J. W. Dudley, Editor

REPRINTED CHAPTERS

Chapters	*Original Titles*	*Authors*	*Sources*
1	Improvement in the chemical composition of the corn kernel	C. G. Hopkins	Illinois Agricultural Experiment Station Bulletin 55, pp. 205-240. 1899.
2	The structure of the corn kernel and the composition of its different parts	C. G. Hopkins L. H. Smith E. M. East	Ibid. 87, pp. 79-112. 1903.
3	Ten generations of corn breeding	L. H. Smith	Ibid. 128, pp. 457-575. 1908.
4	The mean and variability as affected by continuous selection for composition in corn	F. L. Winter	*Journal of Agricultural Research* 39(6):451-476. 1929.
5	Fifty generations of selection for oil and protein in corn	C. M. Woodworth E. R. Leng R. W. Jugenheimer	*Agronomy Journal* 44:60-65. 1952.
6	Nitrogen fractions of the component parts of the corn kernel as affected by selection and soil nitrogen	E. O. Schneider E. B. Earley E. E. DeTurk	*Agronomy Journal* 44:161-169. 1952.
7	Results of long-term selection for chemical composition in maize and their significance in evaluating breeding systems	E. R. Leng	*Zeitschrift fur Pflanzenzuchtung* 47:67-91. 1962. (By permission)
8	Genetic variability after 65 generations of selection in Illinois high oil, low oil, high protein, and low protein strains of *Zea mays* L.	J. W. Dudley R. J. Lambert	*Crop Science* 9:179-181. 1969.

Redaction and retouching were kept to a minimum, especially in tables, figures, and references, for reasons of historical accuracy, practicality, and economy.

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9 Seventy Generations of Selection for Oil and Protein Concentration in the Maize Kernel

J. W. DUDLEY, R. J. LAMBERT, and D. E. ALEXANDER

The photos show individual and rows of corn plants. This breeding program, conducted at the University of Illinois, has been the subject of experimental studies on the selection for oil and protein in the maize kernel for more than seventy generations. An individual corn plant is shown at left, and part of a breeding field is shown below.

1

Improvement in the Chemical Composition of the Corn Kernel

C. G. HOPKINS

INTRODUCTION

The many different uses which are made of corn and the enormous value of the crop to the United States in general, and to the state of Illinois in particular, may certainly be deemed sufficient reason for investigating the possibility of making improvements in the chemical composition of this important grain. The nature of any desired improvement will, of course, depend upon the use which is to be made of the crop produced. For example, if corn is grown for the manufacture of starch, glucose-sugar, syrup, or alcohol, it is desirable that the grain contain a high percentage of carbohydrates, and that the percentages of its other chief constituents, protein and fat, should be reduced as much as possible. If corn is to be used as feed for growing animals or manufactured into corn flour for human food, a higher percentage of protein will certainly increase its value. If it is to be used chiefly for fattening stock, perhaps an increased percentage of fat would be an improvement.

That the chemical composition of corn can be changed seems reasonably probable from the changes which have been produced in some other plants—notably in the sugar beet.

Preliminary Study

Before the work reported in this bulletin could be begun, it was necessary to make a chemical study of the corn plant, and to devise methods for conducting experiments with the object of improving the composition of the grain. It is known that the mineral content of plants can be changed to some extent by the addition to the soil of mineral materials in a form readily available to the plant, but that the temporary change thus effected would have any appreciable hereditary tendency seems very unlikely. The method of procedure which seemed most promising is based upon the common method of making improvement in animals, namely, selecting the best examples of the desired type and breeding successively and under the best conditions from that stock, retaining from each generation only the highest types obtained. This is practically the method by which the sugar content of certain varieties of beets has been increased from less than 5% to 12% or even to 16%. A small portion of a beet is analyzed and, if it is found to be sufficiently rich in sugar, the beet is then set out as a "mother," or seed, beet. From the seed produced beets are grown and another selection of seed beets is made on the basis of their sugar content. But the kernel of corn is not sufficient in quantity to make a complete chemical analysis by any practical method, and certainly the same kernel could not be used for analysis and also for seed.

Early in the year 1896 the writer began a special study of the chemistry of corn. Although, in the latter part of that year, all of the analytical records of the work were destroyed by fire, some valuable knowledge of the subject had been obtained. Among the important facts which the results obtained indicated were: 1) that the ear of corn is approximately uniform throughout in the chemical composition of its kernels, 2) that there is a wide variation in the chemical composition of different ears of the same variety of corn.

That these conclusions are correct has been fully shown by some more recent work of which the data have already been published in detail in Bulletin No. 53, pages 150 to 157.

Plan of Experiments

The uniformity of the individual ear of corn makes it possible to determine very approximately the composition of the grain by analyzing a sample consisting of a few rows of kernels. The remainder of the kernels on the ear may then be planted if desired. The wide variation in composition between different ears is a very important factor in the work of selecting seed, as a starting point is thus furnished in each of the several lines of desired improvement. The general plan of the experiments to improve the composition of corn was to make analyses of samples from a large number of ears, select for seed those ears which were found to contain a high percentage of a desired constituent, plant in an isolated field (to avoid cross fertilization from other corn), and grow the crop under as good field conditions as possible.

From the crop obtained a large number of ears are selected, and samples of each ear are analyzed, seed being taken, as before, from those ears which are found to be highest in the percentage of the constituent which it is desired to increase. Each year this process is repeated.

While it may require 10 or 20 years' work to enable one to form a very definite opinion as to the extent to which it is possible to influence the chemical composition of corn, it is believed that the data and results thus far obtained may be of practical and scientific interest.

General Explanation

All work reported in this bulletin was done upon a single variety of corn, commonly known as Burr's White. It has been grown for several years by this Station with precautions to keep the variety pure and distinct.

The analytical methods employed have been described in detail in Bulletins 43 and 53 of this Station. They are based upon the methods of the Association of Official Agricultural Chemists.

By the term ash is meant the mineral matter which remains after the organic matter is burned.

Protein consists of the nitrogenous organic matter. It is the chief constituent necessary to the growth and repair of the animal body.

The fat is the material extracted from corn by ether. It is practically pure corn oil.

The carbohydrates consist chiefly of starch, but include also the small amounts of other allied substances found in corn, as sugar, fiber, and pentosans.

Unless otherwise stated, all results are reported on the basis of dry matter, or water-free substance.

For more complete explanations of technical terms, or information concerning the uses of food constituents, the reader is referred to the appendix to Bulletin No. 43.

SELECTION OF SEED CORN BY ANALYSIS

From the 1896 crop of Burr's White corn grown upon the Experiment Station farm, two bushels (163 ears) of good, sound ear corn suitable for seed were taken. From each ear a sample consisting of three rows of kernels, lengthwise of the ear, was taken for analysis. The data obtained from the analysis of the 163 samples appear in Table 1.

Plans were made to carry on four separate experiments to change the chemical composition of corn: 1) to increase the protein content, 2) to decrease the protein content, 3) to increase the fat content, 4) to decrease the fat content. It is of course manifest that, if the percentages of protein and and fat are increased, the percentage of carbohydrates is decreased, and vice versa. From the lot of 163 ears, 4 different sets of seed corn were selected on the basis of chemical composition.

1. A set of 24 ears whose percentage of protein was comparatively high.
2. A set of 12 ears each of which contained a low percentage of protein.
3. A set of 24 ears high in fat content.
4. A set of 12 ears low in fat content.

Table 1. Composition of corn from 163 different ears.

Corn No.	Ash.	Protein.	Fat.	Carbo-hydrates.	Corn No.	Ash.	Protein.	Fat.	Carbo-hydrates.
76	1.70	10.05	4.77	83 48	131	1.47	10.49	4.86	83 18
77	1.45	10.42	5.24	82.89	132	1.55	11.13	4.55	82.77
78	1.55	11.00	4.90	82.55	133	1.39	11.13	4.10	83.38
79	1.62	10.89	4.88	82.61	134	1.30	10.85	4.45	83.40
80	1.63	11.50	4.58	82.29	135	1.37	11.29	4.53	82.81
81	1.47	11.49	4.26	82.78	136	1.59	11.43	5.10	81.88
82	1.39	11.78	4.83	82.00	137	1.47	11.61	4.41	82.51
83	1.17	9.08	4.05	85.70	138	1.36	11.36	4.53	82.75
84	1.51	12.79	4.25	81.45	139	1.57	9.81	5.23	83.39
85	1.46	11.76	4.94	81.84	140	1.34	10.53	4.18	83.95
86	1.50	12.07	4.61	81.82	141	1.45	12.42	4.51	81 62
87	1.59	12.40	4.74	81.27	142	1.37	9.31	4.82	84.50
88	1.35	9.34	4.84	84.47	143	1.29	11.33	4.49	82.89
89	1.61	10.71	4.70	82.98	144	1.42	11.39	4.99	82.20
90	1.55	9.90	4.97	83.58	145	1.45	8.25	4.81	85.49
91	1.56	10.68	4.91	82.85	146	1.47	11.29	4.83	82.41
92	1.46	12.96	3.97	81.61	147	1.26	12.21	4.49	82.04
93	1.48	11.80	4.80	81.92	148	1.54	11.94	4.74	81.78
94	1.74	11.89	4.55	81.82	149	1.36	11.29	4.08	83.27
95	1.55	10.49	5.51	82.45	150	1.44	11.71	4.03	82.82
96	1.60	11.10	4.38	82.92	151	1.40	9.31	4.96	84.33
97	1.59	11.84	4.96	81.61	152	1.41	11.90	4.09	82.60
98	1.39	10.23	5.51	82.87	153	1.35	12.51	5.19	80.95
99	1.42	8.40	4.91	85.27	154	1.42	11.13	5.02	82.43
100	1.65	12.28	4.76	81.31	155	1.44	11.05	4.53	82.98
101	1.30	10.08	4.86	83.76	156	1.39	11.74	4.14	82.73
102	1.49	11.83	4.51	82.17	157	1.46	10.02	4.88	83.64
103	1.44	11.25	4.78	82.53	158	1.45	10.66	4.51	83.38
104	1.54	11.82	4.43	82.21	159	1.48	11.53	4.65	82.34
105	1.37	12.36	4.84	81.43	160	1.43	11.50	4.83	82.24
106	1.33	11.15	5.21	82.31	161	1.47	11.11	4.93	82.49
107	1.33	9.47	4.97	84.23	162	1.48	12.09	5.61	80.82
108	1.30	11.04	4.67	82.99	163	1.29	10.78	5.09	82.84
109	1.45	10.82	5.65	82.08	164	1.30	9.36	4.34	85.00
110	1.60	12.81	5.21	80.38	165	1.47	10.50	4.75	83.28
111	1.31	10.76	4.13	83.80	166	1.65	11.29	3.84	83.22
112	1.26	10.48	4.54	83.72	167	1.37	9.58	4.72	84.33
113	1.10	9.30	4.38	85.22	168	1.49	10.94	4.34	83.23
114	1.33	9.12	4.10	85.45	169	1.60	11.79	4.22	82.39
115	1.29	10.41	4.17	84.13	170	1.36	11.06	4.39	83.19
116	1.10	8.38	4.88	85.64	171	1.44	11.18	5.75	81.63
117	1.42	9.95	4.23	84.40	172	1.45	12.28	3.99	82.28
118	1.44	11.40	5.02	82.14	173	1.39	10.14	4.35	84.12
119	1.55	12.38	4.62	81.45	174	1.30	10.19	5.22	83.29
120	1.39	9.97	4.42	84.22	175	1.40	12.68	5.29	80.63
121	1.36	10.09	4.82	83.73	176	1.37	9.86	4.73	84.04
122	1.36	10.31	5.25	83.08	177	1.48	13.06	4.93	80.53
123	1.34	9.68	4.01	84.97	178	1.37	10.93	4.76	82.94
124	1.44	11.87	4.61	82.08	179	1.32	11.87	5.03	81.78
125	1.34	10.73	4.53	83.40	180	1.39	11.27	4.55	82.79
126	1.49	13.87	5.72	78.92	181	1.47	9.66	4.21	84.66
127	1.43	11.53	4.31	82.73	182	1.37	10.97	3.94	83.72

(Continued)

128	1.33	11.64	4.57	82.46	183	1.54	10.32	5.46	82.68
129	1.36	11.25	4.16	83.23	184	1.44	10.68	4.89	82.99
130	1.35	11.86	5.01	81.78	185	1.42	9.33	4.49	84.76
186	1.48	10.78	4.74	83.00	213	1.53	12.40	4.75	81.32
187	1.28	10.49	4.44	83.79	214	1.58	10.22	4.43	83.77
188	1.53	13.10	5.51	79.86	215	1.45	9.22	4.60	84.73
189	1.32	9.58	5.63	83.47	216	1.42	10.27	4.35	83.96
190	1.25	11.50	4.95	82.30	217	1.32	9.39	4.83	84.46
191	1.29	11.19	4.31	83.21	218	1.40	9.74	4.71	84.15
192	1.51	11.49	4.07	82.93	219	1.37	9.92	4.32	84.39
193	1.36	9.47	4.51	84.66	220	1.43	9.63	5.23	83.71
194	1.50	11.47	4.65	82.38	221	1.32	10.33	5.01	83.34
195	1.54	11.09	4.37	83.00	222	1.41	12.34	4.57	81.68
196	1.30	9.44	3.95	85.31	223	1.49	10.58	4.64	83.29
197	1.26	11.20	4.46	83.08	224	1.52	11.36	4.63	82.49
198	1.44	10.23	4.53	83.80	225	1.33	9.15	4.55	84.97
199	1.29	10.64	4.67	83.40	226	1.36	10.31	5.08	83.25
200	1.39	10.13	4.84	83.64	227	1.46	12.63	5.15	80.76
201	1.38	9.64	5.22	83.76	228	1.41	12.16	4.12	82.31
202	1.39	11.26	4.96	82.39	229	1.36	11.04	4.52	83.08
203	1.26	10.48	4.59	83.67	230	1.43	12.10	4.29	82.18
204	1.66	12.57	4.82	80.95	231	1.33	10.95	4.60	83.12
205	1.46	10.71	5.36	82.47	232	1.52	12.76	4.10	81.62
206	1.34	10.27	4.65	83.74	233	1.40	9.75	4.14	84.71
207	1.25	11.09	4.27	83.39	234	1.39	10.78	4.76	83.07
208	1.48	12.05	4.78	81.69	235	1.58	9.97	5.27	83.18
209	1.48	10.22	4.30	84.00	236	1.40	10.18	6.02	82.40
210	1.45	11.16	4.75	82.64	237	1.47	11.16	5.13	82.24
211	1.48	10.44	4.21	83.87	238	1.60	11.42	5.20	81.78
212	1.27	9.75	4.12	84.86					

OUTLINE OF EXPERIMENTS

In the spring of 1897 the four sets of corn which had been selected were planted on four different fields, or plots, each of which was fairly well isolated from other corn fields in order to avoid cross fertilization by corn of different chemical composition. For convenience these four plots are called: 1) high-protein plot, 2) low protein plot, 3) high-fat plot, 4) low-fat plot. Invariably the seed planted in each row was all taken from a single ear; so that the high-protein plot, for example, contained 24 rows planted with seed from the 24 ears selected for that purpose.

In the high-protein and high-fat plots the seed containing the very highest percentage of the desired constituent was planted in the middle rows, the remainder of the seed being planted in approximately uniform gradation to either side. In the low-protein and low-fat plots the seed containing the very lowest percentages of protein and fat, respectively, was planted in the middle rows. This arrangement may be clearly seen by referring to the tables.

By planting plots of both high-protein and low-protein corn, or of both high-fat and low-fat corn, results may be obtained which show the influence of selected seed, as independent and distinguishable from the effects due to the influence of the season.

The plots were given ordinary cultivation, and a good crop of corn was grown on each. When the corn was harvested a set of 10 good ears was selected from each row, excepting from some outer rows. From some of the middle rows duplicate sets of 10 ears each were taken from the same row, as will be seen from the tables, the analytical data from such rows being given in duplicate in all cases. Two rows of kernels (lengthwise of the ear) were taken from each of the 10 ears and mixed to form a composite sample to represent the good corn grown on each row.

EXPERIMENTS TO INFLUENCE THE PROTEIN CONTENT OF CORN

The results from the experiments to change the percentage of protein in corn will be first considered. The tables are arranged to show the percentage of protein in the dry matter of the seed planted and the crop produced. For reference, the Station laboratory serial numbers of all samples analyzed are also given (see Tables 2 and 3).

It is observed that the average composition of the corn from the high-protein plot shows a protein content of 11.10%; while 10.55 is the average percentage of protein in the corn from the low-protein plot, indicating that the difference, .55%, may be ascribed to the influence of the seed selection. On account of the plan, or order, in which the seed corn was arranged in the plots, that is, with the corn of highest protein content in the central rows of the high-protein plot, and the corn of lowest protein content in the central rows of the low-protein plot, we might expect to find a somewhat wider av-

Table 2. Protein in corn planted and harvested on high-protein plot in 1897.

Plot row No.	Corn planted.		Corn harvested	
	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.
1	94	**11.89**	270	**9.61**
2	86	**12.07**	275	**11.07**
3	230	**12.10**	280	**10.94**
4	213	**12.40**	285	**11.48**
5	100	**12.28**	290	**10.85**
6	119	**12.38**	295	**11.64**
7	227	**12.63**	300	**11.46**
8	153	**12.51**	305	**11.57**
9	175	**12.68**	310	**11.17**
10	84	**12.79**	315	**11.14**
11	110	**12.81**	320	**11.16**
12	126	**13.87**	325 330	**11.60** **11.31**
13	92	**12.96**	335 340	**11.07** **11.44**
14	177	**13.06**	345 350	**10.89** **10.67**
15	188	**13.10**	355 360	**10.34** **11.48**
16	232	**12.76**	365	**11.05**
17	87	**12.40**	370	**10.75**
18	204	**12.57**	375	**10.86**
19	105	**12.36**	380	**11.07**
20	141	**12.42**	385	**10.88**
21	172	**12.28**	390	**11.73**
22	222	**12.34**	395	**10.76**
23	147	**12.21**	400	**11.30**
24	208	**12.05**	405	**11.53**
Plot averages		**12.54**	......	**11.10**

Table 3. Protein in corn planted and harvested on low-protein plot in 1897.

Plot row No.	Corn planted.		Corn harvested.	
	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.
1	151	**9.31**		
2	114	**9.12**	410	**10.55**
3	83	**9.08**	415	**10.89**
4	225	**9.15**	420	**10.26**
5	116	**8.38**	425	**10.10**
6	145	**8.25**	430 435	**10.73** **9.90**
7	99	**8.40**	440 445	**10.36** **10.20**
8	215	**9.22**	450	**9.89**
9	185	**9.33**	455	**10.24**
10	164	**9.36**	460	**11.20**
11	113	**9.30**	465	**12.24**
12	193	**9.47**		
Plot averages.		**9.03**	......	**10.55**

erage difference in protein content if we consider only the corn grown on the central half of each plot. From rows 7 to 18 of the high-protein plot we find the average protein content of the corn produced to be 11.12%, while 10.21 is the average percentage of protein in the corn from rows 4 to 9 of the low-protein plot, thus showing an average difference of .91%.

From each set of 10 ears from the 1897 crop, four of those which appeared most suitable for seed corn were reserved for further use. These amounted to 112 ears from the high-protein plot and 48 ears from the low-protein plot. From each of these ears a sample consisting of three or four rows of kernels was taken for analysis, only protein and dry matter being determined. Tables 4 and 5 give the percentage of protein in the dry matter of these samples. The laboratory numbers of these samples of single ears afford ready reference to the row of the plot in which they grew and to the seed from which they were grown. Thus, to each four ears are given the four numbers immediately following the number given to the composite sample of 10 ears from the same row. For example, the composite sample from 10 ears from row 1, high-protein plot, 1897, is given No. 270, as will be seen by reference to Table 2; and the four samples of single ears from the same row are numbered 271, 272, 273, and 274 (Table 4). Table 2 also shows that

Table 4. Protein in samples of 112 ears of corn grown on high-protein plot in 1897.

Corn No.	Protein, per cent.	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.
271	8.82	306	12.33	341	11.65	376	10.47
272	8.42	307	12.39	342	11.35	377	10.92
273	11.60	308	9.64	343	10.60	378	9.32
274	8.34	309	9.93	344	12.16	379	12.28
276	12.83	311	10.65	346	11.63	381	9.31
277	10.46	312	11.05	347	12.26	382	11.00
278	9.95	313	9.89	348	8.76	383	12.23
279	10.96	314	10.22	349	10.69	384	11.99
281	12.62	316	11.08	351	11.39	386	12.10
282	10.43	317	10.29	352	10.59	387	9.20
283	9.87	318	11.72	353	9.65	388	9.76
284	11.58	319	8.76	354	9.83	389	9.18
286	10.97	321	11.43	356	8.63	391	12.46
287	11.08	322	10.94	357	11.08	392	11.14
288	10.23	323	11.18	358	11.39	393	10.03
289	12.99	324	11.55	359	9.12	394	13.27
291	11.52	326	13.62	361	11.63	396	9.94
292	10.44	327	10.99	362	9.98	397	11.78
293	11.92	328	11.07	363	10.45	398	11.30
294	11.25	329	9.18	364	11.89	399	11.08
296	11.11	331	11.40	366	12.01	401	11.23
297	12.07	332	12.24	367	9.51	402	10.92
298	13.58	333	10.06	368	11.43	403	9.72
299	11.68	334	11.02	369	11.76	404	11.14
301	10.80	336	10.78	371	11.75	406	10.44
302	12.26	337	11.28	372	9.46	407	12.72
303	11.20	338	11.09	373	11.17	408	12.80
304	11.97	339	12.85	374	8.67	409	11.17

these ears grew from corn No. 94, which contained 11.89% of protein in the dry matter; so that the complete pedigree of each ear is kept from the beginning of these experiments.

While, as has already been shown, the protein content of corn from the high-protein plot averages higher than that from the low-protein plot, attention is called to the wide variation in the percentage of protein in corn from different ears grown in a single season, in the same plot of ground, and from seed of nearly uniform protein content. This is especially marked in the ears from the low-protein plot (Table 5). For example, corn No. 458 contains 8.22% of protein and grew from seed No. 185 which contained 9.33% of protein; while corn No. 466 contains 13.98% of protein and grew from seed No. 113 whose protein content was 9.30%.

Of course the pedigree of the individual ears used for seed in 1897 was not known, and possibly some variations may be due to hereditary influences, but it seems probable that the wide variations are caused principally by local differences of soil conditions. Some efforts to obviate this difficulty are discussed farther on.

In order to retain hereditary influences the seed for the high-protein plot for 1898 was all selected from corn which grew from seed of high protein content the previous year. On this account corn with high protein content from the low-protein plot was rejected for seed. Likewise seed for 1898 for the low-protein plot was selected only from corn which grew upon that plot in 1897.

In planting the corn in 1898, the same general plan of the previous year was followed. Good crops of corn were grown. Sets of ten ears each were taken from each row, duplicate sets being taken from some rows, as will appear in the tables. Composite samples to represent each row were made, as before, by taking two rows of kernels from each of the ten ears.

Tables 6 and 7 give the percentage of protein in the seed planted and in the crop produced in each row of the plots.

It will be seen that the average protein content of the corn from the high-protein plot for 1898 is 11.05% while 10.55 is the average percentage from the low-protein plot, making a difference of .50%. The difference between the averages becomes .70% if we consider only the central half of each plot. These results are nearly the same obtained in 1897.

In order to avoid local differences in soil conditions, another plot of ground was planted in 1898 with corn of known protein content. For want of a better name this is called the "Mixed-protein Plot." It contained 5 rows of 10 hills each, or 50 hills. In each hill were planted four kernels of corn of which two were high and two were low in protein content. The kernels were so arranged in the hill that the stalk of corn produced by each could be known. When the crop was harvested 8 to 10 ears from both the high-protein seed and the low-protein seed were taken from each row. By taking 2 rows of kernels from each ear 10 composite samples were made of which 5

Table 5. Protein in samples of 48 ears of corn grown on low-protein plot in 1897.

Corn No.	Protein, per cent.	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.
411	11.37	426	11.46	441	10.25	456	10.16
412	11.47	427	8.29	442	10.28	457	10.22
413	11.36	428	10.19	443	11.40	458	8.22
414	11.15	429	9.69	444	9.34	459	11.92
416	8.88	431	10.98	446	8.84	461	11.61
417	9.26	432	9.67	447	11.27	462	10.85
418	11.62	433	9.91	448	9.05	463	10.04
419	10.43	434	12.85	449	8.95	464	11.68
421	9.60	436	9.38	451	10.80	466	13.98
422	9.93	437	10.03	452	10.07	467	12.55
423	12.45	438	10.97	453	12.13	468	13.89
424	10.43	439	9.28	454	10.04	469	12.19

Table 6. Protein in corn planted and harvested on high-protein plot in 1898.

Plot row No.	Corn planted.		Corn harvested.		Plot row No.	Corn planted.		Corn harvested.	
	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.		Corn No.	Protein, per cent.	Corn No.	Protein, per cent.
1	384	11.99	820	11.18	13	298	13.58	960	11.74
2	366	12.01	830	10.86				970	11.42
3	386	12.10	840	10.64	14	289	12.99	980	11.42
4	347	12.26	850	11.26				990	11.20
5	332	12.24	860	11.61	15	276	12.83	1000	11.34
6	306	12.33	870	11.24	16	407	12.72	1010	10.77
7	391	12.46	880	11.26	17	307	12.39	1020	11.03
8	281	12.62	890	10.80	18	379	12.28	1030	10.96
9	408	12.80	900	10.55	19	302	12.26	1040	10.47
10	339	12.85	910	10.92	20	344	12.16	1050	10.33
11	394	13.27	920	11.06	21	383	12.23	1060	11.58
			930	10.67	22	297	12.07	1070	9.78
12	326	13.62	940	11.17	23	304	11.97	1080	10.72
			950	12.48	24	364	11.89	1090	10.95
Plot averages							12.49	....	11.05

Table 7. Protein in corn planted and harvested on low-protein plot in 1898.

Plot row No.	Corn planted.		Corn harvested.		Plot row No.	Corn planted.		Corn harvested.	
	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.		Corn No.	Protein, per cent.	Corn No.	Protein, per cent.
1	421	9.60	1100	10.92	7	427	8.29	1170	10.43
2	444	9.34	1110	11.00				1180	11.14
3	417	9.26	1120	11.03	8	416	8.88	1190	10.68
4	449	8.95	1130	10.06	9	448	9.05	1200	11.16
5	446	8.84	1140	9.83	10	439	9.28	1210	9.93
6	458	8.22	1150	10.26	11	436	9.38	1220	10.27
			1160	10.19	12	432	9.67	1230	10.83
Plot averages							9.06	......	10.55

represent the corn grown in the 5 rows from high-protein seed, and the other 5 represent the corn produced in the same rows from low-protein seed.

Table 8 shows the protein content of the seed planted and of the samples taken from the crop harvested.

The results show that in every row the high-protein seed produced corn with a higher protein content than that produced by the low-protein seed. The average protein content of the corn from the high-protein seed is 11.71%, while 10.46 is the average percentage of protein in the corn from the low-protein seed. This makes an average difference of 1.25%.

In order to obtain more detailed information from this plot, 22 pairs of ears were taken from 22 hills, one ear from each pair having grown from high-protein seed and the other from low-protein seed. The protein in the corn from each ear was determined, and the results are given in Table 9.

The average protein content of the 22 ears from high-protein seed is 11.11%, while 10.15 is the average percentage found in the ears grown from low-protein seed, showing a difference of .96% to be attributed to the influence of the seed selection. In 6 of the 22 pairs the ear from low-protein seed contains more protein than the ear from high-protein seed, these six differences varying from .21% in hill 3 to 3.32% in hill 21. In 16 hills the variation follows the order of the seed, the greatest difference being 4.41% in hill 20.

Table 8. Protein in corn planted and harvested on mixed-protein plot in 1898.

Plot row No	Corn planted.		Corn harvested		Corn planted.		Corn harvested.	
	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.
1	408	**12.80**	698	**11.24**	446	**8.84**	697	**9.72**
2	326	**13.62**	700	**11.75**	458	**8.22**	699	**11.04**
3	407	**12.72**	702	**12.10**	427	**8.29**	701	**10.09**
4	304	**11.97**	704	**11.65**	416	**8.88**	703	**10.89**
5	364	**11.89**	706	**11.81**	448	**9.05**	705	**10.58**
Plot averages............				**11.71**	....			**10.46**

Table 9. Protein in corn from 44 ears grown on the mixed-protein plot in 1898.

Hill No.	From high-protein seed.		From low-protein seed.		Hill No.	From high-protein seed.		From low-protein seed.	
	Corn No.	Protein, per cent.	Corn No.	Protein, per cent.		Corn No.	Protein, per cent.	Corn No.	Protein, per cent.
1	708	**8.89**	707	**10.68**	12	730	**12.12**	729	**9.96**
2	710	**9.11**	709	**8.03**	13	732	**12.20**	731	**12.06**
3	712	**10.17**	711	**10.38**	14	734	**8.85**	733	**9.82**
4	714	**9.88**	713	**10.42**	15	736	**11.55**	735	**10.23**
5	716	**11.64**	715	**9.45**	16	738	**11.56**	737	**11.19**
6	718	**12.28**	717	**8.64**	17	740	**11.49**	739	**8.63**
7	720	**11.23**	719	**9.34**	18	742	**10.04**	741	**10.49**
8	722	**12.39**	721	**10.21**	19	744	**9.33**	743	**7.99**
9	724	**12.39**	723	**10.68**	20	746	**13.55**	745	**9.14**
10	726	**10.23**	725	**8.73**	21	748	**11.49**	747	**14.81**
11	728	**12.24**	727	**11.32**	22	750	**11.73**	749	**11.16**
Averages.							**11.11**	....	**10.15**

It should be stated that owing to cross fertilization no seed corn was selected from this plot. Table 9 offers some good illustrations of the wide variation in the chemical composition of different ears of corn grown from seed of the same variety, of the same composition, during the same season, and in the same soil. Compare, for instance, the corn grown in hills 20 and 21. The corn from high-protein seed shows a difference of 2.06% of protein in favor of hill 20, while the corn from low-protein seed is 5.67% higher in protein in hill 21. Between hills 19 and 21 a difference of soil is indicated by all results obtained, and the corn from the high-protein seed is only 2.16% higher in protein in hill 21 than in hill 19, while a difference of 6.82% appears in the corn from the low-protein seed.

It is evident that this apparent individuality of each particular corn plant will admit of much further study. The most probable explanation which has occurred to the writer is, that the roots of the plant which produces the corn of highest protein content push into the surrounding soil somewhat in advance of the roots of the other plants in the hill and are thus enabled to take up the larger part of the available supply of nitrogen. However, the marked differences frequently observed among different animals of exactly the same breeding lead one to question if the variation in the supply of food materials will entirely explain this individuality of the corn plant. Incidentally it may be stated that the writer has found different ears of good sound Burr's White corn varying from 7.50 to 16.11% of protein in the dry matter. The fact that one good ear of corn has been produced with a protein content above 16% is a promise of the possibility of improving corn in that direction. This belief is strengthened by the experimental results thus far obtained at this Station. A summary of these results will be found at the end of this bulletin.

In Bulletin No. 53, pages 138-141, are quoted some results of combined chemical and mechanical study of the corn kernel. These results show that the protein in the corn kernel is contained principally in the glutenous layer surrounding the main body of the kernel. This layer is very thin at the crown of the kernel, but quite thick at the sides. The germ, in the center of the tip end of the kernel is also rich in protein, although the entire germ constitutes only about 12% of the kernel. The starchy portion, lying between the germ and the glutenous layer and occupying also the center of the crown end of the kernel, consists almost entirely of carbohydrates, although the glutenous layer contains also a large percentage of carbohydrates.

On the basis of this knowledge of the general structure of the corn kernel and chemical composition of its several parts, the writer has made some investigations as to the possibility of selecting corn of high protein content and of low protein content by purely mechanical means, and has found that such a method is both possible and practicable.

By making cross sections and longitudinal sections of several kernels from an ear of corn, one can judge with a very satisfactory degree of accuracy whether the corn is rich or poor in protein.

The illustration (Fig. 1) here shown was made from a photograph taken of the corn kernels and sections with a magnification of three diameters. At the left are two sections and a whole kernel from corn No. 945, containing 14.92% of protein. The sections and whole kernel at the right are from corn No. 1104, containing 7.76% of protein. About one-fourth of the kernel was cut off from the tip end in making the cross sections. In the longitudinal sections the tip end of the kernel points upward to the right.

It will be seen that in the cross sections the white, starchy layer nearly disappears in the high-protein corn but becomes very prominent in the low-protein corn. In the longitudinal sections this difference is also apparent, the white starch in the high-protein corn being confined almost entirely to the crown end of the kernel, while in the low-protein corn it extends into the tip end in considerable amount. The germ in the high-protein corn is somewhat larger. This is also indicated by the depressions in the whole kernels.

As an experiment a number of ears of both high and low content of protein were mixed together and then separated by mechanical examination. It was found that by examining only one or two kernels from each ear the separation could be made with very few errors.

In order to make a more practical test 318 ears of corn were examined.

Figure 1. Left: Corn No. 945; Protein 14.92%.
Right: Corn No. 1104; Protein, 7.76%.

The protein content of the ears in the entire lot did not vary as much as would ordinarily be the case, because 34 of the ears highest in protein and 26 of those lowest in protein had already been removed from this lot of corn. From what remained, however, 18 ears were picked out by mechanical examination as possessing the physical characteristics which indicate a comparatively high content of protein, 15 ears which appeared to be low in protein being selected at the same time. Table 10 shows the results in detail, the percentage of protein in the corn from each ear being given as previously determined by chemical analysis.

The average protein content of the 18 ears selected for high-protein corn is 11.38%, while 9.83 is the average percentage of protein in the 15 ears selected for low-protein corn. Only one ear (No. 3) selected for high-protein corn contains less than 9.83%, and a single ear (No. 1) also selected for low protein corn contains more than 11.38%.

Table 11 shows the results obtained in picking out corn by mechanical examination from a lot which contained corn of only very high or very low protein content.

It will be seen that no errors were made in selecting corn of high protein content, while three mistakes occurred in picking out low-protein corn. It may be stated, however, that these separations were commonly made upon the examination of but one kernel from each ear, and in no case were more than two kernels from an ear examined.

Table 10. Actual protein content of corn selected by mechanical examination.

Ear No.	Corn selected for		Ear No.	Corn selected for		Ear No.	Corn selected for	
	High protein.	Low protein.		High protein.	Low protein		High protein.	Low protein.
	Per cent.	Per cent.		Per cent	Per cent.		Per cent.	Per cent.
1	**11.47**	**11.48**	7	**11.64**	**9.11**	13	**11.87**	**11.27**
2	**12.04**	**9.06**	8	**11.22**	**10.25**	14	**10.21**	**9.36**
3	**9.69**	**9.90**	9	**11.97**	**8.63**	15	**11.71**	**10.25**
4	**11.78**	**9.15**	10	**11.94**	**9.63**	16	**11.59**	
5	**11.65**	**9.67**	11	**10.96**	**8.61**	17	**12.31**	
6	**11.38**	**10.11**	12	**10.83**	**10.95**	18	**10.54**	
Averages..							**11.38**	**9.83**

Table 11. Actual protein content of corn separated by mechanical examination.

Ear No.	Corn selected for		Ear No.	Corn selected for		Ear No.	Corn selected for	
	High protein.	Low protein.		High protein	Low protein.		High protein.	Low protein.
	Per cent.	Per cent.		Per cent.	Per cent.		Per cent.	Per cent.
1	**13.03**	**13.46**	6	**12.83**	**12.39**	11	**12.78**	**8.32**
2	**12.88**	**8.82**	7	**13.25**	**8.57**	12	**12.97**	**7.85**
3	**14.92**	**13.05**	8	**12.98**	**9.02**	13	**13.04**	**8.58**
4	**14.05**	**8.95**	9	**13.04**	**7.85**	14	**16.08**	**7.50**
5	**12.97**	**7.76**	10	**12.82**	**8.29**	15	**12.30**	**8.62**

The examination consists in simply cutting cross sections and longitudinal sections from the kernels with a pocket knife and judging as to the combined amount of glutenous layer and germ in relation to the quantity of the white starchy matter, the observation being made with the naked eye. Some difficulties are met in attempting to form a correct opinion as to whether a kernel is rich or poor in protein. For instance, the disposition of the white starchy matter is not strictly uniform in all kernels. It sometimes happens that the tip end of the kernel contains but very little white starch and a cross section near that end would indicate a high protein content, but at the crown end there may be an excessive proportion of starch and the kernel as a whole be low in protein. For this reason it is important that both cross sections and longitudinal sections be made before judgment is taken. Another difficulty is caused by the great variation in the size of kernels of corn from different ears. A very large kernel, for example, may show a considerable quantity of white starch, extending even to the tip of the kernel, and yet contain a high percentage of protein.

In making the selections given in Tables 10 and 11, the time given to each ear was about a half minute, and it is not assumed that the writer possesses any special skill in judging the comparative sizes of small areas or surfaces, the chief point involved in making these examinations. Indeed, it seems but fair to suppose that the average corn grower could, with some practice and care, make a better selection. In fact, the selections here shown

Table 12. Average weights of kernels from 24 ears of high-protein corn.

Ear No.	Corn No.	Kernel, ave. wt.	Protein, per cent.	Ear No.	Corn No.	Kernel, ave wt.	Protein, per cent.
1	1045	**.470**	12.35	13	951	**.410**	14.25
2	1039	**.290**	12.39	14	895	**.330**	13.46
3	851	**.355**	12.48	15	962	**.400**	13.25
4	1096	**.370**	12.74	16	984	**.430**	13.12
5	826	**.390**	12.83	17	864	**.310**	13.04
6	961	**.340**	12.97	18	1011	**.405**	12.99
7	1062	**.395**	13.03	19	976	**.320**	12.98
8	845	**.440**	13.05	20	1004	**.420**	12 88
9	953	**.355**	13.21	21	871	**.375**	12.82
10	985	**.375**	13.34	22	904	**.405**	12.55
11	959	**.360**	14.05	23	885	**.325**	12.45
12	945	**.370**	14.92	24	1055	**.295**	12.37
General average						**.372**	

Table 13. Average weights of kernels from 16 ears of low-protein corn.

Ear No.	Corn No.	Kernel, ave. wt.	Protein, per cent.	Ear No.	Corn No.	Kernel, ave. wt.	Protein, per cent.
1	1159	**.385**	9.02	9	1104	**.410**	7.76
2	1188	**.355**	8.83	10	1165	**.355**	7.85
3	1211	**.305**	8.66	11	1225	**.280**	8.32
4	1149	**.290**	8.62	12	1131	**.310**	8.58
5	1139	**.345**	8.57	13	1173	**.335**	8.63
6	1167	**.340**	8.29	14	1219	**.360**	8.82
7	1133	**.360**	7.85	15	1151	**.350**	8.90
8	1144	**.275**	7.50	16	1184	**.335**	8.95
General average....................................						**.337**	

were made upon material which was at hand and for the purpose of showing the feasibility of the method, rather than the extent to which it may be carried.

The question whether the size of the corn kernel bears any special relation to the percentage of protein it contains was investigated. Tables 12 and 13 give the average weights in grams of the air-dry kernels from 40 different ears which were used in 1899 as seed for the high-protein and low-protein plots. The percentage of protein in the dry matter is also shown.

The weight of kernels is shown to vary from .290 to .470 g in the high-protein corn, and from .275 to .410 g in the low-protein corn, with a difference between the general averages of .035 g. It is evident that the actual weight of the kernel gives very little, if any, indication as to the percentage of protein which it contains (see Figs. 2 and 3).

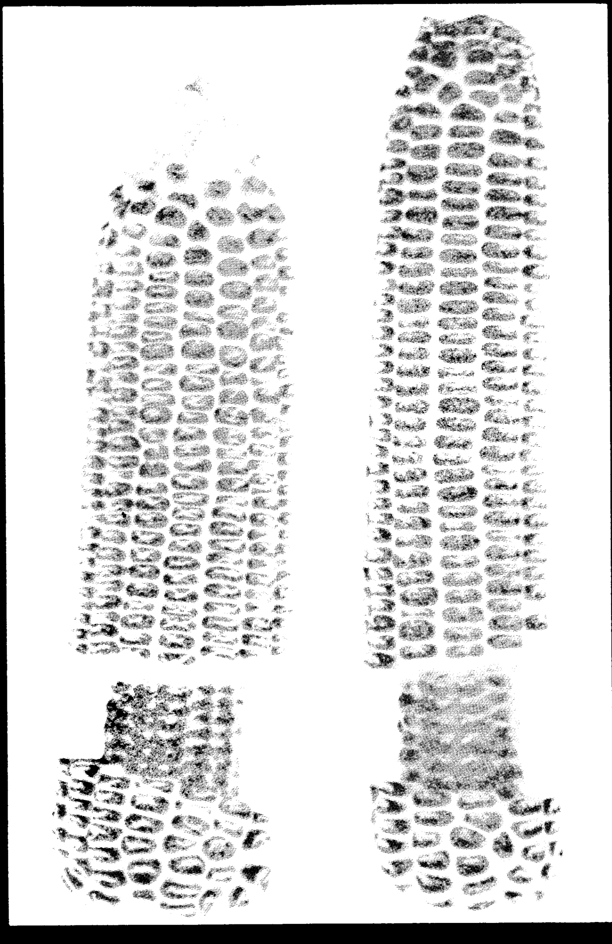

Figure 2. Left: Corn No. 945; Protein, 14.92%; Kernel wt (avg), .370 g.
Right: Corn No. 1104; Protein, 7.76%; Kernel wt (avg), .410 g.

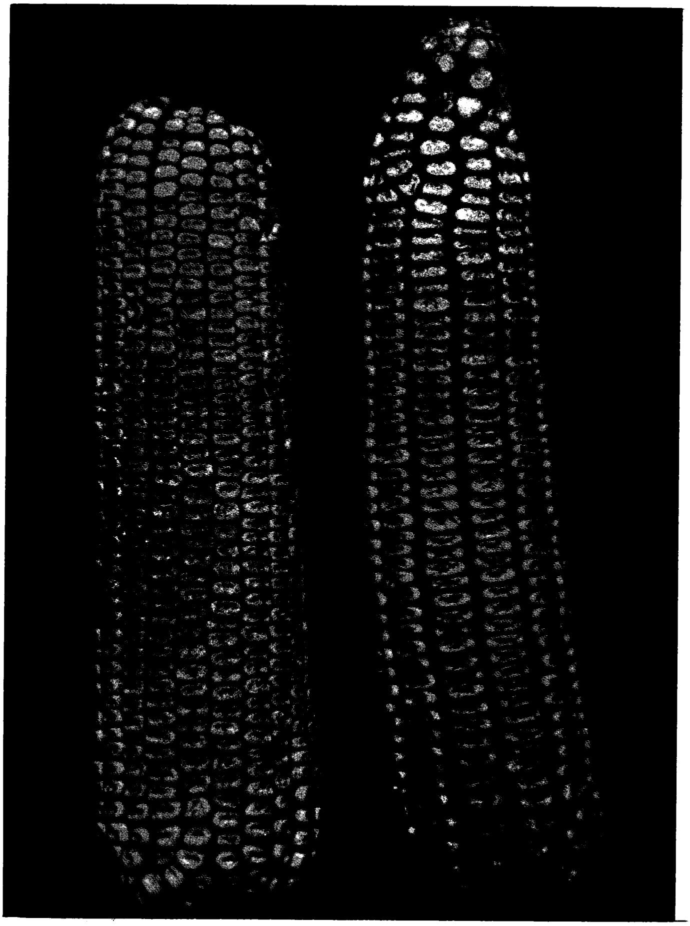

Figure 3. Left: Corn No. 951; Protein, 14.25%; Kernel wt (avg), .410 g.
Right: Corn No. 1149; Protein, 8.62%; Kernel wt (avg), .290 g.

EXPERIMENTS TO INFLUENCE THE FAT CONTENT OF CORN

From the lot of 163 ears of corn from the 1896 crop, the analyses of which are given in Table 1, seed was selected for the high-fat and low-fat plots for 1897, as already explained (under "Outline of Experiments"). Tables 14 and 15 show the percentage of fat in the seed planted and in the crop produced.

These results indicate that the fat content of corn is influenced very markedly by selecting seed according to its percentage of fat. The average fat content of the seed for the high-fat plot is 5.33%, while 4.04 is the average percentage in the seed for the low-fat plot. This shows an average difference in the seed for the two plots of 1.29% of fat. The difference between

Table 14. Fat in corn planted and harvested on high-fat plot in 1897.

Plot row No.	Corn planted.		Corn harvested.		Plot row No.	Corn planted.		Corn harvested.	
	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.		Corn No.	Fat, per cent.	Corn No.	Fat, per cent.
1	118	**5.02**			13	171	**5.75**	520	**4.99**
2	163	**5.09**			14	162	**5.61**	525	**4.84**
3	136	**5.10**	470	**4.43**	15	98	**5.51**	530	**5.23**
4	220	**5.23**	475	**4.74**	16	205	**5.36**	535	**4.70**
5	201	**5.22**	480	**4.77**	17	122	**5.25**	540	**4.47**
6	174	**5.22**	485	**4.65**	18	139	**5.23**	545	**4.81**
7	77	**5.24**	490	**4.50**	19	235	**5.27**	550	**4.38**
8	183	**5.46**	495	**4.53**	20	106	**5.21**	555	**4.80**
9	95	**5.51**	500	**4.98**	21	238	**5.20**	560	**4.58**
10	189	**5.63**	505	**4.75**	22	237	**5.13**	565	**4.46**
11	109	**5.65**	510	**5.40**	23	154	**5.02**		
12	236	**6.02**	515	**4.65**	24	144	**4.99**		
Plot averages							**5.33**	......	**4.73**

Table 15. Fat in corn planted and harvested on low-fat plot in 1897.

Plot row No.	Corn planted.		Corn harvested.		Plot row No.	Corn harvested.		Corn planted.	
	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.		Corn No.	Fat, per cent.	Corn No.	Fat, per cent.
1	149	**4.08**			7	182	**3.94**	595	**4.01**
1	152	**4.09**	570	**3.96**	8	123	**4.01**	600	**4.06**
3	150	**4.03**	575	**4.21**	9	228	**4.12**	605	**3.97**
4	192	**4.07**	580	**4.31**	10	133	**4.10**	610	**4.05**
5	196	**3.95**	585	**4.05**	11	212	**4.12**	615	**4.22**
6	166	**3.84**	590	**3.79**	12	156	**4.14**		
Plot averages							**4.04**	......	**4.06**

the average fat contents of the crops from the two plots is .67%, the average from the high-fat plot being 4.73 and from the low-fat plot 4.06% of fat. There is a difference of .79% of fat between the averages of the two crops, if we consider only the central half of each plot. It is noteworthy that the lowest percentage of fat in the corn from any row of the high-fat plot, namely, 4.38% in row 19, is higher than the highest percentage obtained from any row in the low-fat plot.

It will be seen that samples were not taken from some of the outer rows in these plots, namely, rows 1, 2, 23, and 24 in the high-fat plot, and rows 1 and 12 in the low-fat plot. From all other rows sets of 10 ears each were taken, the results here given being obtained by the analysis of the composite sample for each row.

From each set of 10 ears from the 1897 crop, 4 ears were taken for individual analysis, a sample of 3 or 4 rows of kernels (lengthwise of the ear) being taken for this purpose. In these samples only fat and dry matter were determined. The system of numbering the samples was the same as that followed in the experiments with the protein content of corn, the multiple of five being given to the composite sample and the next four numbers to the samples of four single ears from the same row. Thus, the composite sample from row 3, high-fat plot, 1897, is numbered 470 (Table 14), and the four samples of individual ears from the same row are numbered 471, 472, 473, and 474 (Table 16).

Eighty samples of single ears from the high-fat plot and 40 from the low-fat plot were analyzed. The percentage of fat in the dry matter is given in Tables 16 and 17.

It will be remembered that extreme variations are common in the protein content of different ears of corn even when grown the same season, from seed of uniform protein content, and in practically the same soil. Such variations do not seem characteristic of the fat content. Of the 80 ears selected from the high-fat plot, only one contained less than 4.20% of fat, while 4.06 is the average percentage of fat in the corn from the low-fat plot. On the other hand no ear from the low-fat plot was found to contain above 4.74%

Table 16. Fat in samples of 80 ears of corn grown on high-fat plot in 1897.

Corn No.	Fat, per cent.	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.
471	**4.44**	491	**4.85**	511	**5.44**	531	**5.04**	551	**4.31**
472	**4.79**	492	**4.38**	512	**5.45**	532	**4.82**	552	**4.33**
473	**4.42**	493	**4.93**	513	**5.49**	533	**4.98**	553	**4.24**
474	**4.59**	494	**4.97**	514	**5.39**	534	**5.27**	554	**4.33**
476	**4.84**	496	**4.26**	516	**4.63**	536	**4.97**	556	**4.93**
477	**4.82**	497	**4.59**	517	**5.26**	537	**4.50**	557	**4.68**
478	**5.39**	498	**4.76**	518	**4.81**	538	**4.92**	558	**4.92**
479	**4.40**	499	**4.45**	519	**4.44**	539	**4.83**	559	**5.12**
481	**5.04**	501	**5.45**	521	**4.98**	541	**4.78**	561	**4.41**
482	**4.87**	502	**4.95**	522	**4.22**	542	**3.60**	562	**4.62**
483	**4.46**	503	**4.64**	523	**4.91**	543	**4.91**	563	**4.95**
484	**5.07**	504	**4.77**	524	**5.68**	544	**5.02**	564	**4.23**
486	**5.03**	506	**4.91**	526	**4.70**	546	**5.20**	566	**4.39**
487	**4.20**	507	**4.69**	527	**5.43**	547	**5.00**	567	**4.20**
488	**4.72**	508	**5.04**	528	**5.12**	548	**4.90**	568	**5.05**
489	**4.86**	509	**4.20**	529	**4.68**	549	**4.81**	569	**4.42**

Table 17. Fat in samples of 40 ears of corn grown on low-fat plot in 1897.

Corn No.	Fat, per cent.	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.
571	**4.00**	581	**4.74**	591	**3.85**	601	**4.68**	611	**3.84**
572	**3.96**	582	**4.69**	592	**3.72**	602	**3.55**	612	**4.08**
573	**3.89**	583	**4.65**	593	**3.38**	603	**3.80**	613	**4.39**
574	**3.83**	584	**4.07**	594	**3.39**	604	**4.42**	614	**3.39**
576	**4.21**	586	**4.21**	596	**4.21**	606	**3.50**	616	**4.08**
577	**4.28**	587	**4.74**	597	**4.22**	607	**4.40**	617	**4.19**
578	**4.18**	588	**3.70**	598	**4.42**	608	**3.90**	618	**4.43**
579	**4.41**	589	**3.85**	599	**4.04**	609	**3.90**	619	**4.68**

of fat, although 60% of the ears from the high-fat plot contained above that percentage of fat, the maximum being 5.68%.

For 1898 the seed for the high-fat plot was from corn which grew on the high-fat plot in 1897, 24 of the 80 ears whose fat content is shown in Table 16 being selected. For the low-fat seed 12 ears were selected from the 40 ears whose percentage of fat is shown in Table 17, all of which were grown from low-fat seed in 1897. The system of planting the highest of the high-fat seed and the lowest of the low-fat seed in the middle rows of the respective plots was followed in 1898. Good crops of corn were grown, and, when harvested, sets of 10 ears each were taken from each row, composite samples to represent each row being made, as before, by taking 2 rows of kernels from each of the 10 ears.

Tables 18 and 19 give the percentage of fat in the composite samples and also in the seed planted in each row.

The average fat content of the corn from the high-fat plot for 1898 is 5.15%, while 3.99 is the average percentage of fat in the corn from the low-

Table 18. Fat in corn planted and harvested on high-fat plot in 1898.

Plot row No.	Corn planted.		Corn harvested.		Plot row No.	Corn planted.		Corn harvested.	
	Corn No.	Fat, per cent.	Corn No.	Fat, per cent,		Corn No.	Fat, per cent.	Corn No.	Fat, per cent.
1	521	4.98	1240	4.86	13	513	5.49	1360	5.21
2	533	4.98	1250	4.74	14	512	5.45	1370	5.44
3	486	5.03	1260	4.94	15	527	5.43	1380	5.48
4	531	5.04	1270	5.17	16	514	5.39	1390	5.26
5	568	5.05	1280	5.36	17	517	5.26	1400	5.55
6	528	5.12	1290	4.79	18	559	5.12	1410	5.23
7	546	5.20	1300	4.87	19	484	5.07	1420	5.06
8	534	5.27	1310	5.20	20	544	5.02	1430	4.89
9	478	5.39	1320	5.16	21	508	5.04	1440	5.00
10	511	5.44	1330	5.25	22	547	5.00	1450	5.10
11	501	5.45	1340	5.21	23	536	4.97	1460	5.05
12	524	5.68	1350	5.63	24	494	4.97	1470	5.21
Plot averages							5.20	......	5.15

Table 19. Fat in corn planted and harvested on low-fat plot in 1898.

Plot row No.	Corn planted.		Corn harvested.		Plot row No.	Corn planted.		Corn harvested.	
	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.		Corn No.	Fat, per cent.	Corn No.	Fat, per cent.
1	589	3.85	1480	3.97	7	593	3.38	1540	3.69
2	574	3.83	1490	4.32	8	606	3.50	1550	3.78
3	592	3.72	1500	4.08	9	588	3.70	1560	3.93
4	602	3.55	1510	3.99	10	603	3.80	1570	4.18
5	594	3.39	1520	3.81	11	611	3.84	1580	4.21
6	614	3.39	1530	3.81	12	591	3.85	1590	4.11
Plot averages							3.65	......	3.99

fat plot, making a difference of 1.16% between the averages, and the difference becomes 1.45% if we consider only the central half of each plot, or 1.56% if only the central third of each plot is considered. The effect of planting the seed in gradation as to fat content from the center rows to either side is decidedly noticeable in the crop. It is only necessary to take averages of the fat content of the composite samples from the high-fat plot in groups of four to obtain a regular gradation in the same order as that of the seed. Thus—

Corn from rows	Fat, average %
1– 4	4.93
5– 8	5.06
9–12	5.31
13–16	5.35
17–20	5.18
21–24	5.09

In the low-fat plot the percentages of fat in the composite samples from the single rows are in regular gradation, if we omit only the outside rows, Nos. 1 and 12. This may be seen in Table 19.

There is some indication of the influence of the season upon the fat content of corn, which becomes apparent by comparing the results obtained in the two different years 1897 and 1898 (see Table 20).

The season of 1897 seems to have favored the production of corn of low fat content, the average percentage of fat in the crop from the low-fat plot being but .02% higher than in the seed, while in the high-fat plot the crop is .60% below the seed in fat content. In 1898 the production of corn high in fat seems to have been favored, the fat content of the crop being only .05% below that of the seed in the high-fat plot and .34 above that of the seed in the low-fat plot.

Table 20. Average percentages of fat in corn planted and harvested in 1897 and 1898.

Season		1897.	1898.
High-fat plot.	**Fat in corn planted**	5.33	5.20
	Fat in corn harvested	4.73	5.15
	Difference	**.60**	**.05**
Low-fat plot.	**Fat in corn planted**	4.04	3.65
	Fat in corn harvested	4.06	3.99
	Difference	**.02**	**.34**

In 1898 a third plot of ground for the study of the fat content of corn was planted. This is called the "Mixed-fat Plot," and was planted after the same plan as the mixed-protein plot. It contained 50 hills arranged in 5 rows of 10 hills each. In each hill two kernels of high-fat corn were planted on one side and two of low fat content on the other. The special object in this work was, of course, to avoid the influence of soil differences. When the crop was harvested composite samples were made of the corn from each side of each row, two rows of kernels from eight to ten ears being used for each composite sample. Table 21 shows the fat content of the seed and of the crop for each side of each row.

The difference between the average fat content of the corn from high-fat seed and that from low-fat seed is 1.11%. The lowest fat content of any composite sample from high-fat seed is 4.66%, and 4.20 is the highest percentage of fat in a composite sample of corn from low-fat seed.

From the mixed-fat plot 27 pairs of ears were taken from 27 hills, one ear in each pair having grown from high-fat seed and the other ear from low-fat seed. Table 22 gives the fat content in the corn from each of these ears.

Table 21. Fat in corn planted and harvested on mixed-fat plot in 1898.

Plot row No.	Corn planted.		Corn harvested.		Corn planted.		Corn harvested.	
	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.	Corn No.	Fat, per cent.
1	546	**5.20**	752	**4.66**	594	**3.39**	751	**3.60**
2	511	**5.44**	754	**4.87**	614	**3.39**	753	**3.86**
3	524	**5.68**	756	**5.38**	593	**3.38**	755	**4.13**
4	513	**5.49**	758	**5.14**	606	**3.50**	757	**4.20**
5	527	**5.43**	760	**5.35**	588	**3.70**	759	**4.06**
Plot averages...				**5.08**	....			**3.97**

Table 22. Fat in corn from 54 ears grown on the mixed-fat plot in 1898.

Hill No.	From high-fat seed.		From low-fat seed.		Hill No.	From high-fat seed.		From low-fat seed.	
	Corn No.	Fat, per cent.	Corn No	Fat, per cent.		Corn No.	Fat, per cent	Corn No.	Fat, per cent.
1	762	**4.05**	761	**3.82**	14	788	**5.03**	787	**4.08**
2	764	**4.42**	763	**3.62**	15	790	**5.57**	789	**3.57**
3	766	**4.65**	765	**3.03**	16	792	**5.32**	791	**4.69**
4	768	**4.90**	767	**3.92**	17	794	**5.75**	793	**3.96**
5	770	**5.16**	769	**3.94**	18	796	**4.95**	795	**4.64**
6	772	**5.06**	771	**3.99**	19	798	**4.79**	797	**4.30**
7	774	**5.13**	773	**4.15**	20	800	**4.59**	799	**4.33**
8	776	**4.95**	775	**3.75**	21	802	**5.56**	801	**3.77**
9	778	**5.59**	777	**3.60**	22	804	**5.34**	803	**4.17**
10	780	**4.10**	779	**4.04**	23	806	**4.92**	805	**4.50**
11	782	**4.49**	781	**3.56**	24	808	**5.91**	807	**3.58**
12	784	**5.25**	783	**4.26**	25	810	**5.86**	809	**4.55**
13	786	**5.65**	785	**4.15**	26	812	**4.59**	811	**3.96**
					27	814	**5.02**	813	**4.23**
Averages..................							**5.06**	......	**4.01**

The average percentage of fat in the 27 ears from high-fat seed is 5.06, while 4.01% is the average fat content of the same number of ears from low-fat seed. It is interesting to note that in the 27 hills there is no instance where the ear of corn from high-fat seed does not contain more fat than the ear grown from low-fat seed in the same hill. The difference in fat content between ears in the same hill varies from .06% in hill No. 10 to 2.33% in hill No. 24.

The ear which grew from low-fat seed in hill No. 3 has the lowest fat content, 3.03%, of any ear of corn which has been analyzed in these experiments. The maximum fat content which has been found in an ear of Burr's White corn up to the present time is 6.71%. It seems reasonable to suppose that these limits may be reached again or exceeded, and possibly by corn in larger amounts than single ears. The experiments upon the fat content of corn are summarized at the end of this bulletin.

The fact that the fat, or oil, of the corn kernel is contained almost entirely in the germ (see Bulletin No. 53, pages 139 and 140) suggested to the writer the possibility of selecting corn, of high or low fat content by mechanical examination of the kernel and judging as to the quantity of germ compared with the remainder of the kernel. It was found that the method is

Figure 4. Left: Corn No. 1338; Fat, 6.08%.
Right: Corn No. 1512; Fat, 3.64%.

possible and rather more satisfactory than the method (already described) of judging the protein content of the corn kernel by mechanical examination, as it is less complicated than the latter.

Figure 4 (made from a photograph taken with a magnification of three diameters) illustrates the difference in corn kernels of about the same size but of very different fat content. The cross sections, shown at the top, were made by cutting off about one-fifth of the kernel from the tip end. In the longitudinal sections the tip end of the kernel points downward to the left. The sections and kernel shown at the left are from an ear of corn (No. 1338) which contains 6.08% of fat. Those at the right are from an ear (No. 1512) containing 3.64% of fat.

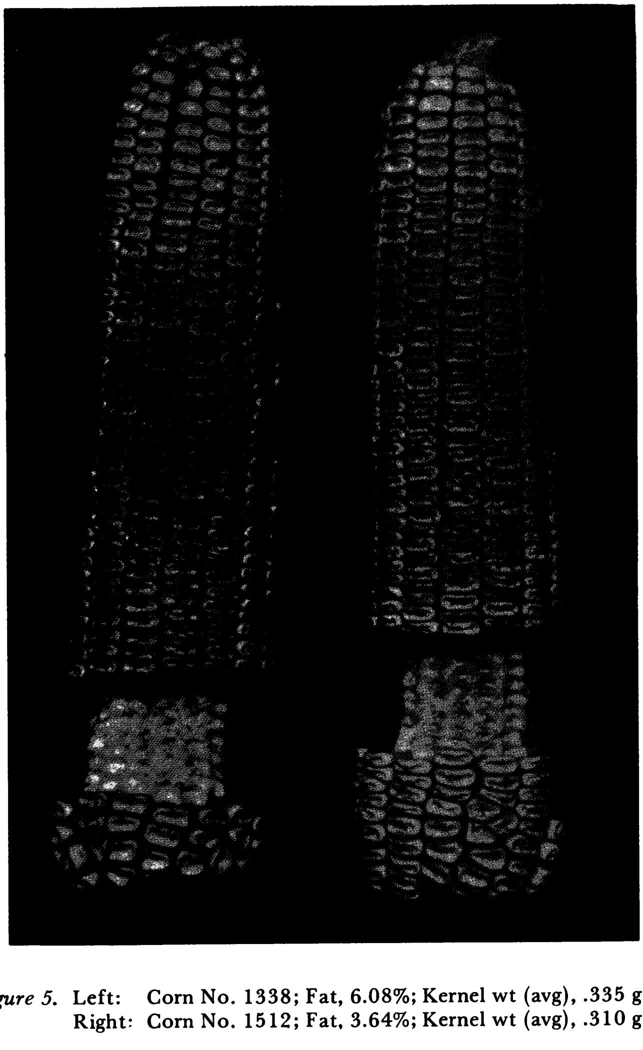

Figure 5. Left: Corn No. 1338; Fat, 6.08%; Kernel wt (avg), .335 g.
Right: Corn No. 1512; Fat, 3.64%; Kernel wt (avg), .310 g.

It will be seen that the germ is larger in the high-fat corn and that it extends nearly the entire length of the kernel, while in the low-fat corn the germ is small and about two-thirds as long as the kernel. Aside from the rather slight difference in the size of the depressions, the general appearance of the whole kernels is about the same whether they are rich or poor in fat content. This is true also of ears with kernels of about the same size. This may be seen from Figure 5 which illustrates the two ears from which were taken the kernels shown in Figure 4, the high-fat ear being at the left and the low-fat at the right.

There is apparently some tendency toward large kernels with ears of corn having a low fat content, and vice versa, indicating that the change in the percentage of fat is brought about, in part, at least, by the absolute increase or decrease of carbohydrates. In other words, in selecting seed with a low percentage of fat, as determined by chemical analysis, the ears chosen will have a tendency not only to small germs but to large kernels. This is illustrated by Tables 23 and 24, which give the average weights in grams of the air-dry kernels from 28 different ears which were used in 1899 as seed for high-fat and low-fat plots. The percentage of fat in the dry matter is also given.

The average weight of the high-fat kernels is .345 g, while the low-fat kernels average .420 g. However, there are wide variations from this apparent tendency.

Table 23. Average weights of kernels from 12 ears of high-fat corn.

Ear No.	Corn No.	Kernel, ave. wt.	Fat, per cent.	Ear No.	Corn No.	Kernel, ave. wt.	Fat, per cent.
1	1413	**.325**	5.83	7	1354	**.295**	6.49
2	1342	**.370**	5.90	8	1476	**.400**	6.34
3	1338	**.335**	6.08	9	1308	**.315**	6.09
4	1314	**.345**	6.28	10	1276	**.405**	5.90
5	1389	**.305**	6.47	11	1379	**.340**	5.89
6	1352	**.340**	6.71	12	1259	**.370**	5.82
General average...						**.345**	

Table 24. Average weights of kernels from 16 ears of low-fat corn.

Ear No.	Corn No.	Kernel, ave. wt.	Fat, per cent.	Ear No.	Corn No.	Kernel, ave. wt.	Fat, per cent.
1	1521	**.490**	3.64	9	1522	**.525**	3.27
2	1559	**.430**	3.63	10	1531	**.380**	3.33
3	1564	**.500**	3.58	11	1545	**.470**	3.35
4	1538	**.370**	3.56	12	1486	**.430**	3.39
5	1504	**.390**	3.38	13	1557	**.500**	3.56
6	1539	**.355**	3.34	14	1543	**.410**	3.59
7	1516	**.390**	3.32	15	1512	**.310**	3.64
8	1529	**.320**	3.22	16	1548	**.450**	3.65
General Average						**.420**	

While the tendency of high-fat corn to small kernels and of low-fat corn to large kernels aids, in a way, in the selection of corn of high or low fat content, the difficulty of judging the percentage of fat by the comparative sizes of germ and kernel is greatly increased by the wide variations in the size of kernels. With kernels of approximately the same size, and with germs of similar shape, as those shown in Figure 4, it is an easy matter to distinguish between high-fat corn and low-fat corn; but frequently a large kernel of low fat content will have a larger germ than a smaller kernel of a higher percentage of fat; or the germs in the kernels from one ear may be short and thick, and from another ear they may be long and slender, the difficulties in the way of forming accurate judgment being thus increased.

To obtain exact data as to the relation between the percentage of fat and the percentage of germ in the corn kernel, the germs were removed from a large number of kernels, the weight of the whole kernel and also of the separated germ being determined and reported on the basis of dry matter, having been dried in hydrogen before being weighed. It was found that after soaking corn kernels in hot water for about 30 minutes the germs are easily removed in the entire state and quite free from other portions of the kernel.

In Table 25 are given the results from 80 kernels of corn, 10 kernels being taken from each of 8 different ears. No. 1354 represents an ear of high fat content, 6.49%, and small kernels, average weight .2652 g. The germs average .0374 g in weight, and amount to 14.11% of the whole kernel. Corn No. 1564 contains 3.58% of fat. The kernels average .4631 g and the germs .0421 g in weight. The germs amount to only 9.10% of the whole kernel although the average weight of the germ is considerably more than in No. 1354. This ear, No. 1564, illustrates a relatively low fat content produced by an absolute increase in carbohydrates. In corn No. 1352, containing 6.71% fat and No. 1529, containing 3.22% fat, the kernels are approximately uniform in size, the former being .3013 and the latter .3181 g in average weight. The germs in the high-fat ear amount to 12.40% of the whole kernels, and to only 8.56% in the low-fat ear. This difference is due to the absolute difference in the size of the germs, the germs from the high-fat kernels being .0373 g and from the low-fat kernels only .0272 g average weight. Nos. 1338 and 1512 are the same ears as are shown in Figure 5, and from which the kernels shown in Figure 4 were taken. The fat content is 6.08 and 3.64% and the percentage of germ 12.01 and 8.28, respectively. Nos. 1259 and 1516 are ears with medium-sized kernels, the average weight being about the same from each ear. The former contains 5.82% of fat and 13.30% of germ, the latter 3.32% of fat and 8.73% of germ, as an average.

It will be seen that the general relation between the percentage of fat and the percentage of germ in the corn kernel is clearly established. Of course there are minor individual differences among the kernels from the same ear, and it is also noted that there is a difference in different ears as to the relation between fat content and germ content. For example, corn No.

Table 25. Weights of corn kernels with weight and percentage of germ.

Kernel No	Kernel, wt. gms.	Germ, wt. gms.	Germ, per cent.	Kernel No.	Kernel, wt. gms.	Germ, wt. gms.	Germ, per cent.
Corn No. 1354.—Fat = 6.49 per cent.				Corn No. 1564.—Fat = 3.58 per cent			
1	.2611	.0411	**15.74**	1	.4478	.0418	**9.33**
2	.2718	.0385	**14.16**	2	.4428	.0446	**10.07**
3	.2633	.0370	**14.05**	3	.4402	.0402	**9.13**
4	.2789	.0382	**13.70**	4	.4943	.0420	**8.50**
5	.2427	.0347	**14.30**	5	.4205	.0405	**9 63**
6	.2859	.0372	**13.01**	6	.4714	.0439	**9.31**
7	.2642	.0362	**13.70**	7	.4968	.0436	**8.78**
8	.2595	.0329	**12.68**	8	.4398	.0361	**8.21**
9	.2567	.0359	**13.99**	9	.4581	.0446	**9.74**
10	.2680	.0422	**15.75**	10	.5188	.0432	**8 33**
Averages,	.2652	.0374	**14.11**	Averages,	.4631	.0421	**9.10**
Corn No. 1352.—Fat = 6.71 per cent.				Corn No. 1529.—Fat = 3.22 per cent.			
1	.3113	.0391	**12.56**	1	.2859	.0250	**8.74**
2	.2872	.0360	**12.53**	2	.2882	.0237	**8.22**
3	.2864	.0340	**11.87**	3	.3533	.0297	**8.41**
4	.2821	.0374	**13.26**	4	.3135	.0265	**8.45**
5	.2667	.0360	**13.50**	5	.3277	.0273	**8.33**
6	.3694	.0442	**11.97**	6	.3417	.0310	**9.07**
7	.3434	.0414	**12.06**	7	.2918	.0257	**8.81**
8	.2682	.0300	**11.18**	8	.3178	.0276	**8.68**
9	.3116	.0402	**12.90**	9	.3273	.0278	**8.49**
10	.2870	.0348	**12.13**	10	.3338	.0280	**8.39**
Averages,	.3013	.0373	**12.40**	Averages,	.3181	.0272	**8.56**
Corn No. 1338.—Fat = 6.08 per cent.				Corn No. 1512.—Fat = 3.64 per cent.			
1	.3268	.0373	**11.41**	1	.2813	.0258	**9.17**
2	.3229	.0385	**11.92**	2	.3156	.0259	**8.21**
3	.3031	.0308	**10.16**	3	.2674	.0206	**7.70**
4	.3122	.0400	**12.81**	4	.2740	.0229	**8.36**
5	.3025	.0349	**11.54**	5	.2805	.0250	**8.91**
6	.3018	.0384	**12.72**	6	.2935	.0232	**7.90**
7	.2963	.0363	**12.25**	7	.3223	.0251	**7.79**
8	.3095	.0361	**11.66**	8	.2752	.0238	**8.65**
9	.2871	.0395	**13.76**	9	.3008	.0233	**7.75**
10	.3424	.0407	**11.89**	10	.2664	.0222	**8.33**
Averages	.3105	.0373	**12.01**	Averages	.2877	.0238	**8.28**
Corn No. 1259.—Fat = 5.82 per cent.				Corn No. 1516.—Fat = 3.32 per cent.			
1	.2719	.0371	**13.64**	1	.3969	.0338	**8.52**
2	.3172	.0390	**12.30**	2	.3293	.0246	**7.47**
3	.3571	.0468	**13.11**	3	.3586	.0364	**10.15**
4	.3602	.0514	**14.27**	4	.3434	.0318	**9.26**
5	.3446	.0482	**13.99**	5	.3401	.0296	**8.70**
6	.3786	.0455	**12.02**	6	.3348	.0283	**8.45**
7	.3453	.0450	**13.03**	7	.3317	.0272	**8.20**
8	.3206	.0454	**14.16**	8	.3096	.0240	**7.75**
9	.3927	.0472	**12.02**	9	.3843	.0340	**8.85**
10	.3304	.0476	**14.41**	10	.3733	.0373	**9.99**
Averages	.3419	.0453	**13.30**	Averages	.3502	.0307	**8.73**

1354 contains 6.49% of fat and 14.11% of germ; while corn No. 1352 contains a higher percentage of fat but a lower percentage of germ. Again the corn of lowest fat content is not quite the lowest in percentage of germ. These minor differences are perhaps due in part, to the varying percentage of fat in the remainder of the kernel, although the variation in the percentage of fat in the germ is doubtless the chief factor in producing such differences. For example, Voorhees found 26.65% of fat in the germs of the corn kernel, while Balland found 39.85%. (See Bulletin No. 53, page 140).

The method of selecting corn of high or low fat content by mechanical examination is similar to that described under the work on the protein content of the corn kernel, excepting that the size of the germ alone as compared with the remainder of the kernel is considered. Judgment is formed by examining with the naked eye the cross sections and longitudinal sections of a few kernels from an ear.

Table 26 shows the results obtained in picking out corn by mechanical examination from a lot which contained corn of only very high or of very low fat content.

In picking out 15 ears for high-fat corn and 15 ears for low-fat corn but one error was made, namely, the fourth ear selected for high-fat corn which really contained a low percentage of fat.

To make a more practical test of the method a miscellaneous lot of corn was examined—in all 272 ears, which varied in fat content from about 3.60 to 5.80%. Twelve ears of very high fat content and sixteen ears of very low fat content had been taken from this lot and used for seed in 1899 (see Tables 23 and 24), otherwise the results would no doubt have been more marked than they are.

From the lot of 272 ears, by mechanical examination 18 ears were selected which appeared to possess a comparatively high fat content, and at the same time 30 ears apparently low in fat were selected. Tables 27 and 28 give the results.

The average fat content of the ears selected for high-fat corn is 5.24%, while 4.13 is the average of that selected for low-fat corn.

If we omit ear No. 2 in Table 27 and ear No. 15 in Table 28, the lowest percentage of fat in the ears selected for high fat content is higher than the highest percentage in the low-fat selection.

Table 26. Actual fat content of corn separated by mechanical examination.

Ear No.	Corn selected for		Ear No.	Corn selected for		Ear No.	Corn selected for	
	High fat.	Low fat.		High fat.	Low fat.		High fat.	Low fat.
	Per cent.	Per cent.		Per cent.	Per cent.		Per cent.	Per cent.
1	**5.90**	**3.56**	6	**6.71**	**3.65**	11	**5.80**	**3.67**
2	**6.08**	**3.59**	7	**6.49**	**3.22**	12	**6.09**	**3.50**
3	**6.28**	**3.63**	8	**5.94**	**3.67**	13	**5.82**	**3.63**
4	**3.65**	**3.58**	9	**5.87**	**3.27**	14	**5.90**	**3.64**
5	**6.47**	**3.32**	10	**6.34**	**3.39**	15	**5.89**	**3.64**

Table 27. Fat content of 18 ears selected by mechanical examination for high-fat corn.

Ear No.	Fat, per cent.	Ear No.	Fat, per cent.	Ear No.	Fat, per cent.	Ear No.	Fat, per cent.	Ear No.	Fat, per cent.
1	**4.94**	5	**5.23**	9	**5.22**	13	**5.27**	17	**4.97**
2	**4.30**	6	**5.58**	10	**5.33**	14	**5.12**	18	**5.21**
3	**5.43**	7	**5.06**	11	**5.55**	15	**5.73**		
4	**5.64**	8	**5.26**	12	**4.99**	16	**5.43**		
Average..									**5.24**

Table 28. Fat content of 30 ears selected by mechanical examination for low-fat corn.

Ear No.	Fat, per cent.	Ear No.	Fat, per cent.	Ear No.	Fat, per cent.	Ear No.	Fat, per cent.	Ear No.	Fat, per cent.
1	**4.01**	7	**4.07**	13	**3.73**	19	**4.52**	25	**4.09**
2	**4.11**	8	**4.20**	14	**3.76**	20	**4.29**	26	**4.27**
3	**3.64**	9	**3.91**	15	**5.21**	21	**3.81**	27	**4.02**
4	**3.67**	10	**4.85**	16	**3.63**	22	**4.39**	28	**3.87**
5	**4.52**	11	**4.35**	17	**4.02**	23	**4.43**	29	**4.00**
6	**3.66**	12	**4.52**	18	**4.55**	24	**3.80**	30	**3.98**
Average..									**4.13**

Table 29. Weight of corn kernels with weight and percentage of germ.

Ear 2. Table 27.—Fat = 4.30 per cent.				Ear 15. Table 28.— Fat = 5.21 per cent.			
Kernel No.	Kernel, wt. gms.	Germ, wt. gms.	Germ, per cent.	Kernel No.	Kernel, wt. gms.	Germ, wt. gms.	Germ, per cent.
1	.3580	.0346	**9.66**	1	.4014	.0450	**11.21**
2	.3670	.0362	**9.86**	2	.3674	.0348	**9.47**
3	.2939	.0277	**9.42**	3	.3975	.0463	**11.65**
4	.2841	.0306	**10.77**	4	.4392	.0410	**9.33**
5	.2969	.0262	**8.82**	5	.4571	.0539	**11.79**
6	.3054	.0326	**10.67**	6	.3541	.0442	**12.48**
7	.3156	.0304	**9.63**	7	.3968	.0377	**9.50**
8	.2866	.0284	**9.91**	8	.4225	.0463	**10.96**
9	.3452	.0354	**10.25**	9	.3850	.0497	**12.91**
10	.3053	.0280	**9.17**	10	.4096	.0464	**11.33**
Averages	.3158	.0310	**9.82**	Averages	.4031	.0445	**11.06**

Ten kernels from each of the two ears just mentioned were taken, and the exact percentage of germ in each kernel determined, in order to ascertain whether these ears were selected because of incorrect judgment or were exceptions to the general rule that the percentage of fat varies with the percentage of germ in the corn kernel. The results are given in Table 29.

While some variations in individual kernels from the same ear exist, it is seen that the corn of 4.30% fat contains 9.82% of germ, while the ear with 5.21% fat contains 11.06% of germ, in the kernel as an average, showing that the judgment was incorrect, having been formed possibly from insufficient data.

SUMMARY OF EXPERIMENTS TO IMPROVE THE CHEMICAL COMPOSITION OF THE CORN KERNEL

All results thus far obtained indicate that it is possible to influence the composition of corn; that by proper selection of seed any of its principal constituents, protein, fat, or carbohydrates, may be increased or decreased.

In 1897 a plot of corn planted with seed containing a high percentage of protein produced a crop containing 11.10% of protein, while 10.55 was the percentage contained in a crop grown from seed of low protein content. By considering only the central half of each plot, the crop from seed high in protein shows 11.12% of protein, and the crop from low-protein seed shows 10.21%.

In 1898 the crop of corn from the seed of high protein content contained 11.05% of protein, while that from low-protein seed contained 10.55%. If only the central half of each plot is considered the results show 11.17% and 10.47% of protein in the corn from the respective seed.

The average protein content of 22 ears of corn grown in 22 different hills from high-protein seed was 11.11%, while 10.15 was the average percentage of protein contained in 22 other ears grown in the same 22 hills from low-protein seed.

Fifty hills of corn were planted with two kernels of high-protein seed and two kernels of low-protein seed in each hill. The average protein content of the crop grown from high-protein seed was 11.71%, while 10.46 was the average percentage in the crop from the low-protein seed.

In the six tests the selection of seed corn of high and low protein content has produced differences in the crops varying from .50 to 1.25% of protein.

In 1897 a plot of corn planted with seed of high fat content produced a crop containing 4.73% of fat, while the crop produced on a plot planted with seed of low fat content contained an average of 4.06%. Considering only the central half of each plot the crop from the high-fat seed contained 4.82% of fat, while that from low-fat seed contained 4.03%.

In 1898 the high-fat seed produced a crop containing 5.15% of fat and the crop from low-fat seed contained only 3.99%. Again by considering only the central half of each plot the percentages of fat in the crops are found to be 5.29 and 3.84, respectively.

The average fat content of 27 ears grown in 1898 in 27 different hills from high-fat seed was 5.06%, while 4.01 was the average percentage of fat contained in 27 other ears grown in the same 27 hills from seed of low fat content.

Fifty hills were planted with two kernels of high-fat seed and two kernels of low-fat seed in each hill. The average fat content of the crop produced from the high-fat seed was 5.08%, while 3.97 was the average percentage in the crop from low-fat seed.

In the six tests the selection of seed of high and low fat content has produced differences in the crops varying from .67 to 1.45% of fat.

The fat content of corn is even more susceptible to the influence of seed selection than is the protein content, doubtless due to the fact that the primary materials from which fat is manufactured, namely, carbon dioxide and water, are usually furnished to the plant in unlimited supply, while the formation of protein is essentially dependent upon the supply of available nitrogen in the soil.

As the percentage of carbohydrates (principally starch in corn) varies inversely with the combined percentages of protein and fat it follows that the carbohydrates are increased in percentage whenever the combined percentage of protein and fat is decreased, and vice versa.

It has been found that the protein content of corn varies chiefly with the proportion of glutenous layer in the kernel and that by mechanical examination of corn kernels this variation in the proportion of glutenous layer can easily be observed with the naked eye.

It has also been found that the fat content of corn varies quite uniformly with the proportion of germ in the kernel and that by mechanical examination this variation in the relative amount of germ in the whole kernel can also be observed with the eye.

By actual trial it has been found both possible and practicable to select corn by mechanical examination with either high or low content of protein, fat, or starch.

While further investigations are necessary, and are in progress, to determine more accurately the best methods and more definitely the possibilities of improvement in the chemical composition of corn, it is here stated, tentatively, that essentially by the methods reported in this bulletin any corn grower will be able to select seed and to breed corn to increase or to decrease the percentage of any one of its three principal chemical constituents.

All experiments reported in this bulletin have been carried on with the one variety of corn, namely, Burr's White. Of course, it is not believed that Burr's White is the best variety for improvement in corn in every one of the several important lines. Indeed it seems highly probable that one variety of corn will be found best adapted to but one line of improvement. We have in progress chemical studies of other varieties of corn, and a considerable amount of data and information has been already acquired, but it is reserved, pending further investigations, for future publication, the special object of this bulletin being to give the data, thus far obtained, indicating the possibility and establishing the fact that the corn kernel may be improved in chemical composition.

It may be stated that improvement in the composition of other parts of the corn plant is under consideration by this Station. Plans are made also to investigate other questions relating to this general subject; such as the effect of changes in the chemical composition of corn upon its digestibility, vitality, yield, etc.

The results obtained in our investigations to improve the composition of corn have suggested the possibility of improving other grains by somewhat similar methods. It seems not improbable that the different grains or kernels produced in a single head of wheat, oats, barley, etc., may be found to be approximately uniform in composition. If so, a method is thus offered for selecting seed according to its chemical composition.

2

The Structure of the Corn Kernel and the Composition of Its Different Parts

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SUMMARY

The investigations reported in this bulletin serve to establish the following facts:

1. The kernel of corn consists of six readily observable and distinctly different physical parts, which are known as 1) the tip cap, 2) the hull, 3) the horny gluten, 4) the horny starch, 5) the white starch, 6) the germ.

2. The tip cap covers the tip or base of the kernel and comprises only about 1.5% of the grain.

3. The hull is the very thin outer coat. It comprises about 6% of the kernel and contains a lower percentage of protein (about 4%) than any other part of the kernel.

4. The horny glutenous part (aleurone layer) lies underneath the hull surrounding the kernel. It comprises from 8 to 14% of the grain (being more abundant in high-protein corn), and it contains from 20 to 25% of protein, being the richest in protein of all the parts of the corn kernel.

5. The horny starchy part is the chief substance in the sides and back of the kernel (the germ face being considered the front of the kernel). This substance comprises about 45% of ordinary corn, but is much more abundant in high-protein corn and less abundant in low-protein. Although rich in starch, it contains about 10% of protein (more in high-protein corn and less in low-protein corn). It contains a greater total amount of protein than any other part of the kernel.

6. The white starchy part occupies the center of the crown end of the kernel and usually partially surrounds the germ. It comprises about 25% of the kernel (less in high-protein corn and more in low-protein corn). It is poor in protein (5 to 8%).

7. The germ occupies the central part of the kernel toward the tip end. It comprises about 11% of the kernel (more in high-oil corn and less in low-oil corn). The germ contains from 35 to 40% of corn oil, or from 80 to 85% of the total oil content of the corn kernel.

8. High-protein corn contains a large proportion of the horny parts (both of the horny glutenous part and the horny starchy part) and a correspondingly smaller proportion of the white starchy part. The horny parts comprise more than 60% of high-protein corn and contain about 80% of the total protein content of very high-protein corn.

9. The value and reliability of the method proposed in previous bulletins by which any farmer can select high-protein seed corn (selecting for a large proportion of horny parts) by a simple mechanical examination of the corn kernels has been fully confirmed by the results which have been frequently obtained and which are now reported in this bulletin.

10. The value of the method proposed for picking out high-oil seed corn by selecting for a large proportion of germ is also fully established.

11. The degree of correlation existing in the corn kernel between the percentage of germ and of protein is very slight and is frequently entirely absent; consequently the proportion of germ in the corn kernel is not a reliable index of its protein content.

12. The composition of the different products obtained from corn by hominy mills, as well as by other factories, serves greatly to emphasize the importance of breeding corn for special purposes.

Note: For more complete details of corn breeding, the reader is referred to Bulletin No. 100, "Directions for the Breeding of Corn, Including Methods for the Prevention of Inbreeding."

INTRODUCTION

The possibility of selecting seed corn for improved chemical composition by a simple mechanical examination of sections of kernels (which any one can easily make with a pocketknife) was clearly established by the experiments reported in Bulletin No. 55, "Improvement in the Chemical Composition of the Corn Kernel"; and the practical value of this method of selecting seed corn for high protein, high oil, or other desirable qualities has been fully confirmed by subsequent investigations and trials by the Experiment Station and by practical seed-corn breeders, as shown in Bulletin No. 82, "Methods of Corn Breeding."

A considerable amount of additional data relating to this matter has been accumulating with the progress of our experiments in corn breeding, and because of the very great importance of this subject to the corn growers and corn breeders of Illinois, and also because of the marked interest which is manifested in this matter both by progressive, practical farmers and by scientific investigators, it has seemed advisable to publish in somewhat greater detail the results of our investigations along this line.

PARTS OF THE CORN KERNEL

There are six distinctly different parts in a kernel of corn, as will be readily seen by reference to Figs. 1 and 2.

1. Tip cap.—This is a small cap covering the tip end of the kernel and

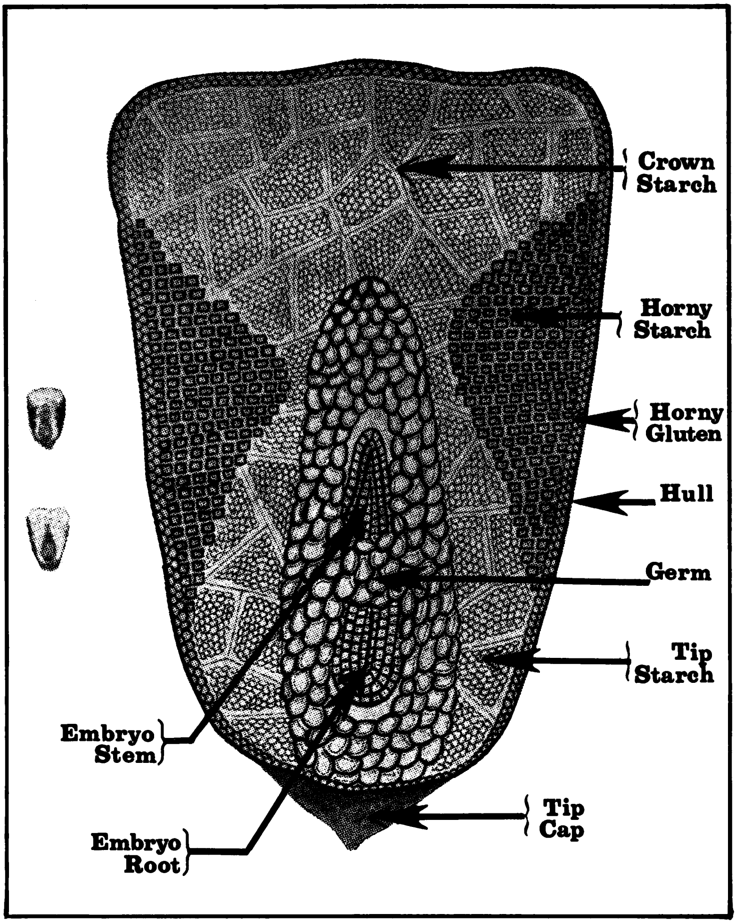

Figure 1. Low-protein corn kernel from drawing (small kernels from photograph).

serving as a protection to the end of the germ. It consists of material somewhat resembling the cob. Occasionally in shelling corn the tip cap remains attached to the cob, leaving the tip end of the germ uncovered, but nearly always it remains on the kernel.

2. Hull.—This is the very thin outer covering of the kernel. It consists largely of carbohydrates, especially fiber or cellulose, although it also contains a small percentage of other constituents.

3. Horny glutenous part.—This part lies immediately underneath the hull. It constitutes a second covering of the kernel, usually much thicker than the hull. For short it is called horny gluten, although, of course, it is not pure gluten. However, it is the richest in protein of any part of the corn kernel, as has been stated in bulletins already published by this station and

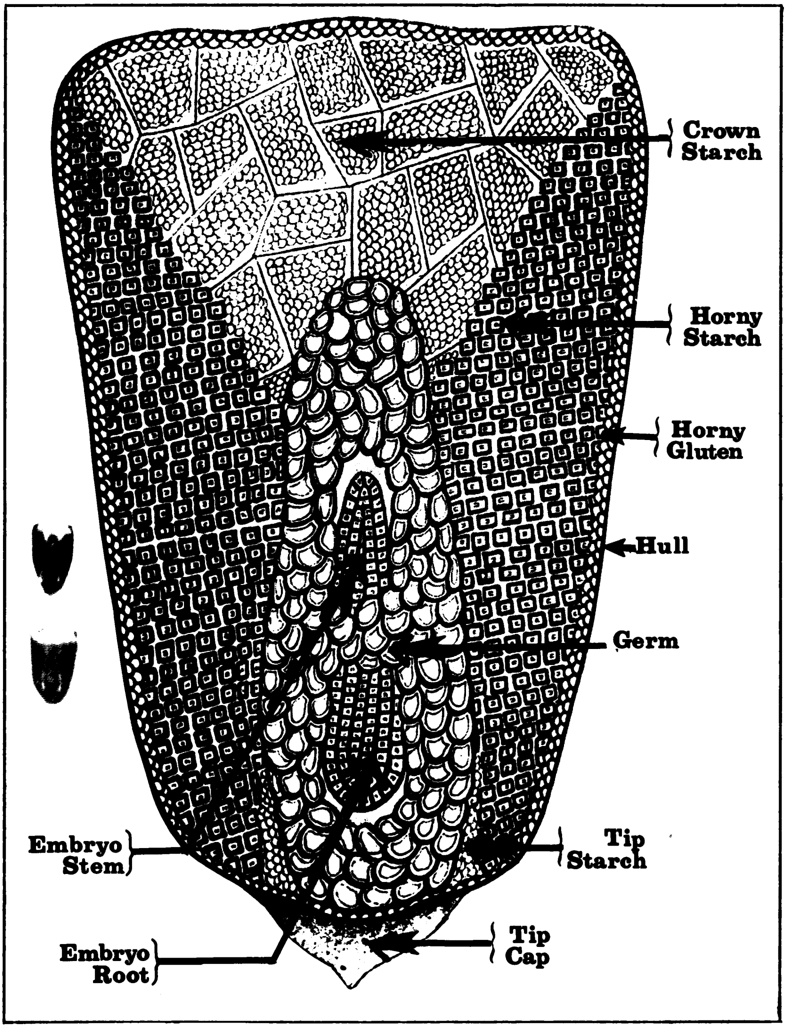

Figure 2. High-protein corn kernel from drawing (small kernels from photograph).

previously by Doctor Voorhees, Director of the New Jersey Experiment Station.

4. Horny starchy part.—This part lies next to the horny gluten, on the back and sides of the kernel. For short it is called horny starch, although it is not pure starch, as it contains considerable amounts of other constituents, especially protein. In an examination of the kernel with the unaided eye, the horny glutenous part and the horny starchy part are not readily distinguished from each other, the line between them being somewhat indefinite

and indistinct. Considered together, these two parts constitute the horny part of the kernel.

5. White starchy part.—This part occupies the crown end of the kernel above the germ and it also nearly surrounds the germ toward the tip end of the kernel. For convenience this material is called white starch, although it is not pure starch, as has been clearly shown in former publications. In some kernels the horny starch extends nearly or quite to the germ (near the middle of the kernel), and thus separates more or less completely the white starch into two parts, which we call crown starch and tip starch.

6. Germ.—The germ occupies the center of the front of the kernel toward the tip end and usually extends about one-half or two-thirds the length of the kernel. Within the body of the germ are the embryo stem, pointing upward toward the crown end, and the embryo root, pointing downward toward the tip of the kernel, both of which are, of course, parts of the germ. These embryo parts within the germ may be easily seen by any one who will carefully shave off the front side of the germ from a kernel of corn. (See small photographic reproduction of sections of kernels of high- and low-protein corn in Figs. 1 and 2.)

MECHANICAL SEPARATION OF THE DIFFERENT PARTS

It is not a difficult matter to obtain very pure samples of each of the above-named parts of the corn kernel, although in making the separations there is of necessity some waste material consisting of a mixture of three different parts: namely, horny gluten, horny starch, and white starch.[1]

By the use of a small, sharp knife any one can make the following separations:

1. Tip cap
2. Hull
3. Horny gluten
4. Horny starch

5a. Crown starch
5b. Tip starch
6. Germ
7. Waste (mixed material)

In making these separations the kernels are first soaked in hot water for 15 or 20 minutes.

The tip cap is then very easily and perfectly separated by simply cutting under one edge and then pulling it off.

The hull is separated without difficulty by peeling it off in strips. It is only necessary to use the knife to start the peeling at the tip end where the hull has been broken by removing the tip cap. With some care the hull can be completely peeled out of the dent in the corn.

The horny gluten is more easily distinguished after the hull is removed. It will be plainly seen that it covers the entire kernel, excepting possibly the exposed part of the germ. The horny gluten is removed by carefully shaving it off with a sharp knife. Adhering particles of starch can be more easily

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[1] As used in this bulletin, the term "starch" is employed in a technical or commercial sense, and not as the name of a definite chemical compound.

separated from the horny gluten after the shavings have been allowed to dry for some time. In scraping off these particles of horny starch or white starch adhering to the shavings, more or less horny gluten will also be scraped off, so that, while we are thus able to obtain a pure, clean sample of the horny gluten, we also obtain some waste material, consisting of particles of horny gluten, horny starch, and white starch.

The germ is next removed, and with care this can be done very perfectly. If any particles of starch adhere to the germ, they can easily be completely removed. After the tip cap, hull, horny gluten, and germ have been removed, the remainder of the kernel, consisting of the horny starch and white starch only, is allowed to dry, and the kernel is broken in two lengthwise.

The crown starch is dug out with the knife as completely as possible without taking any of the horny starch.

The tip starch is next removed in the same manner as the crown starch.

The horny starch from each side usually remains in a solid piece. This is now carefully scraped to remove all adhering particles of white starch or horny gluten, the scrapings being carefully saved and added to the waste material.

By this method of separation we obtain eight different products, including the waste material. Seven of these products, excepting the crown starch and tip starch, are pure samples of distinctly different parts of the corn kernel. Although the crown and tip starch of course belong to the white starch, they are kept separate because found in different places, frequently being entirely separated in the kernel, although more commonly there is some white starch continuous from crown to tip.

COMPOSITION OF THE DIFFERENT PARTS

Table 1 shows the percentage of these eight different products, or parts, and the percentage composition of each part, also the percentage composition of the whole corn, for each of three different ears of corn. Ear No. 1 is corn of comparatively low protein content[2]. Ear No. 2 has about the usual protein content of ordinary corn. Ear No. 3 is high-protein corn. About 200 g (nearly .5 lb) of kernels from each ear were separated into the different parts, and each part was then weighed and analyzed separately, another sample of the corn from each ear being analyzed to give the composition of the whole corn. (All results are given on the water-free basis.)

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[2] It should be understood that Ear No. 1 (9.28% protein) and Ear No. 3 (12.85% protein) do not represent extremes in protein content, indeed, in our breeding of corn for low protein we have produced good ears containing less than 6.5% of protein, and in our high-protein field we have produced corn containing over 16% of protein. In extremely low-protein corn the percentage of horny part is very much less than in Ear No. 1, and in extremely high-protein corn the tip white starch is frequently almost entirely wanting and the crown white starch very greatly reduced, both being replaced by the horny part, as shown in the drawings and also in the actual photographs of sections of kernels shown beside the drawings in Figs. 1 and 2.

Table 1. Percentage of different parts and percentage composition of each part.

Names of parts	Percent of whole	Composition of parts			
		Protein, percent	Oil, percent	Ash, percent	Carbohydrates, percent
Ear No. 1 (low in protein)					
Tip caps	1.20	7.36	1.16	.91	90.57
Hulls	5.47	4.97	.92	.82	93.29
Horny gluten	7.75	19.21	4.00	.92	75.87
Horny starch	29.58	8.12	.16	.18	91.54
Crown starch	16.94	7.22	.19	.32	92.27
Tip starch	10.93	6.10	.29	.29	93.31
Germs	9.59	19.91	36.54	10.48	33.07
Mixed waste	18.53	9.90	1.06	.61	88.43
Whole corn	.....	9.28	4.20	1.41	85.11
Ear No. 2 (medium in protein)					
Tip caps	1.46	8.83	2.30	1.11	87.76
Hulls	5.93	3.96	.89	.79	94.36
Horny gluten	5.12	22.50	6.99	1.72	69.09
Horny starch	32.80	10.20	.24	.24	89.32
Crown starch	11.85	7.92	.17	.24	91.67
Tip starch	5.91	7.68	.39	.31	91.62
Germs	11.53	19.80	34.81	9.90	35.46
Mixed waste	25.40	11.10	1.23	.57	87.10
Whole corn	.....	10.95	4.33	1.55	83.17
Ear No. 3 (high in protein)					
Tip caps	1.62	4.64	1.99	1.87	91.50
Hulls	6.09	3.84	.76	1.10	94.30
Horny gluten	9.86	24.58	4.61	1.74	69.07
Horny starch	33.79	10.99	.22	.21	88.58
Crown starch	10.45	8.61	.52	.37	90.50
Tip starch	6.23	7.29	1.36	.60	90.75
Germs	11.93	19.56	33.71	10.00	36.73
Mixed waste	20.03	12.53	1.15	.61	85.71
Whole corn	.....	12.85	5.36	1.67	80.12

A careful study of Table 1 reveals some very interesting and useful facts regarding the structure of the corn kernel and the composition of the different parts. It is certainly an interesting fact that there are so many different parts in a kernel of corn, and it is exceedingly useful to be able by a mechanical examination of corn not only to pick out high-protein corn or high-oil corn, as one may desire, but even to separate the several distinctly different parts from one another by purely mechanical means—to separate, for example, the horny gluten, containing (in the high-protein ear) nearly 25% of protein, and then the white starchy parts, with only 7 or 8% of protein; or the germs, containing about 35% of oil and 10% of ash, and then the horny starchy part, containing less than .25% of either oil or ash.

The hulls contain about 4% of protein and are clearly the poorest in protein of any part of the kernel, the next poorest being the tip caps and white starchy parts, containing about 7 or 8%, the tip starch being slightly poorer than the crown starch. The horny starch is richer in protein than the white starch, especially in the medium- and high-protein corn, where the difference amounts to more than 2%, the horny starchy part containing from 10 to 11% of protein. The protein content of the germs is very uniform in the different ears, although the poorest germs are found in the high-protein corn and the richest in the low-protein corn, the variation being from 19.56 to 19.91%. The horny gluten is the richest in protein of any part of the kernel in both ordinary and high-protein corn, as was pointed out several years ago by Doctor Voorhees (1), Director of the New Jersey Experiment Station, and as we have quoted in previous publications of the Illinois Experiment Station. In the high-protein corn the protein content of the horny gluten amounts to 24.58%; in the low-protein corn it is slightly less than that of the germ.

It is plainly seen that the oil in corn is very largely in the germ, although the horny gluten also contains a considerable percentage, the germ containing about 35% of oil and the horny gluten about 5%. Both the horny and the white starch are exceedingly poor in oil, averaging about .25%, if we disregard the tip starch in Ear No. 3, which appears to have absorbed some oil directly from the germ, which it adjoins and partially surrounds. The hulls contain slightly less than 1% of oil and the tip caps slightly more than 1%, and it is quite possible that this oil may have been obtained, in part at least, by absorption from the horny gluten and germ. Indeed, it seems highly probable that practically all of the true oil in the corn kernel is originally deposited in the germ and horny gluten, and that the small percentage, or mere trace, which is found in the other parts is largely obtained by absorption. That such absorption actually does occur is definitely proved by the fact that the percentage of oil in hominy and hominy products increases with the age of the corn used in the milling. (Hominy consists largely of the horny starch, with more or less adhering white starch.)

It may be of interest to state in this connection that in 1866 Haberlandt (2) discovered with the microscope that the germ of the corn kernel contains a large amount of oil. He observed no oil in the remaining portions of the kernel. By chemical analysis Lenz (2) found, however, that after the germs were removed the remaining portion of the kernel contained 1.57% of oil. These results were fully confirmed by Doctor Atwater (3), who found 1.63% of oil in the corn after removing the germs and adjoining material, although neither Lenz nor Atwater appear to have ascertained that the horny gluten (the aleurone layer) contains the chief percentage of oil outside of the germ.

By further reference to Table 1 it will be observed: 1) that the germ contains about 10% of ash or mineral matter; 2) that this is about 10 times the average percentage of ash contained in the other parts; and 3) that the percentage of ash in the different parts varies with the percentage of oil, to quite a noticeable degree.

Of course, the percentage of carbohydrates (starch, cellulose, pentosans, etc.) varies inversely as the sum of the other constituents, being about 35% in the germ, 70% in the horny gluten, and from 90 to 95% in the other principal parts.

The marked degree of uniformity in the entire percentage composition of the germs from each of these three ears, whether low-protein, medium-protein, or high-protein, seems especially noteworthy. The percentage of protein varies only from 19.56 to 19.91; the oil from 33.71 to 36.54; the ash from 9.90 to 10.48; and the carbohydrates from 33.07 to 36.73. It will also be noted that the percentages both of protein and of oil are lower in the germs from high-protein corn than in those from the low-protein corn, although the differences are not marked.

MATHEMATICAL DISTRIBUTION OF WASTE

It will be borne in mind that in making the mechanical separations, in order to obtain each of the seven different parts in pure condition, unmixed with any other part, there was necessarily some waste product. This waste substance amounted to about 20% of the whole. As has already been explained, this mixed waste material consists of only three distinctly different parts—horny gluten, horny starch, and white starch (from crown and tip), the other three parts—tip caps, hulls, and germs—being easily separated completely and in pure form.

By a simple computation the mixed waste material can be distributed among the respective parts of which it is composed, provided we may be allowed to make the assumption (which is approximately the truth) that the horny starch and the white starch are present in the waste material in the same proportions as they are in the pure, separated portions. Any error which might be introduced by following this assumption would have but little effect, because in composition the horny starch and the white starch are not very markedly different (the protein differs by from 2 to 3%); and also because the total amount of waste material to be distributed is only from one-third to one-half the sum of the separated horny starch and white starch.

It will be observed (see Table 1) that the mixed waste is always richer in protein than the horny starch, thus showing that, besides horny starch and white starch, it also contains more or less horny gluten, which, of course, we know to be the fact.

If in 100 g of corn we let

x equal the number of g of tip starch,
Bx equal the number of g of crown starch,
Cx equal the number of g of horny starch,
y equal the number of g of horny gluten, and
S equal the sum of these four parts; then

$$x + Bx + Cx + y = S.$$

Now if we let
a equal the % of protein in the tip starch,
b equal the % of protein in the crown starch,
c equal the % of protein in the horny starch,
d equal the % of protein in the horny gluten, and
s equal the number of g of protein in all of these four parts; then

$$ax + bBx + cCx + dy = s.$$

Thus we have two equations with which to solve for x and y, which are the only unknown quantities, B and C being factors which can be obtained by dividing the percentages of separated crown starch and horny starch, respectively, by that of tip starch, and S being the sum of the separated tip starch, crown starch, horny starch, horny gluten, and mixed waste, as given in Table 1; and a, b, c, d being the respective percentages of protein in the four separated materials, tip starch, crown starch, horny starch, and horny gluten, and s being the total number of grams of protein contained in these four separated parts and in the mixed waste, all of which data are also given in Table 1.

PHYSICAL COMPOSITION OF THE CORN KERNEL

From the above computations we obtain the results given in Table 2, which gives the total percentages of each of the seven different parts contained in the corn kernel (counting crown starch and tip starch as two parts), and with no waste material.

It will be observed that the percentages of horny gluten, horny starch, and germs are noticeably higher in the high-protein corn than in the low-protein corn; while the opposite is true with the white starch, the percentages of crown starch and tip starch being markedly higher in the low-protein corn than they are in the high-protein corn. It is noteworthy that the horny gluten in high-protein corn not only contains a higher percentage of protein than the germs, but that the proportion of horny gluten in the kernel equals or exceeds that of the germs. The only discrepancies appearing in Table 2 are the low percentage of horny gluten and the high percentage of horny starch in Ear No. 2. Otherwise the percentages of parts in the medium-

Table 2. Total percentages of the different parts of the corn kernel.

Names of parts	Ear No. 1, (low-protein)	Ear No. 2, (medium-protein)	Ear No. 3, (high-protein)
Tip caps	1.20	1.46	1.62
Hulls	5.47	5.93	6.09
Horny gluten	11.61	8.51	13.32
Horny starch	37.15	47.08	44.89
Crown starch	21.26	17.01	13.88
Tip starch	13.71	8.48	6.28
Germs	9.59	11.53	11.93
Total	99.99	100.00	100.01

Table 3. Percentages of the different parts of the corn kernel as commonly observed in mechanical examination for seed-corn selection.

Names of parts	Ear No. 1, (low-protein)	Ear No. 2, (medium-protein)	Ear No. 3, (high-protein)	Average percent
Tip caps	1.20	1.46	1.62	1.43
Hulls	5.47	5.93	6.09	5.83
Horny part	48.76	55.59	58.21	54.19
White starch	34.96	25.49	22.16	27.54
Germs	9.59	11.53	11.93	11.02
Total	99.98	100.00	100.01	100.00

protein ear are always intermediate between those in the other two ears, as would be expected. Even these discrepancies disappear if the two horny parts be added together and considered as one part, as is done in the practical work of selecting seed corn for higher protein content by mechanical examination, as will be seen by referring to Table 3.

In this table the crown starch and tip starch are also added together and the sum recorded as white starch. The increase in the amount of horny part (from 48.76 to 58.21%) and the decrease in white starch (from 34.96 to 22.16%) as we pass from the low-protein to the high-protein corn, is plainly apparent.

DISTRIBUTION OF CHEMICAL CONSTITUENTS

Table 4 shows the location or complete distribution of the chemical constituents among the seven different physical parts of the corn kernel. In other words, this table represents the separation of 100 g (or 100 lb) of corn into seven different structural or physical parts, and the subsequent division of each of these parts into the four chemical constituents, protein, oil, ash, and carbohydrates.

The complete data shown in Table 4 are presented especially for the benefit of farmers who are corn breeders, and also for the benefit of the manufacturers of corn products. The agreement between the sum of the separate determinations and the direct determinations of the same constituent in the whole corn is very satisfactory, considering that these results are obtained by computation from the analyses of nine different materials, including the whole corn. The greatest difference is well within the limit of unavoidable error in sampling and analytical determination. A careful study of this table will reveal some interesting and valuable facts. For example, it will be seen that in 100 lb of the low-protein corn the horny gluten contains only 2.23 lb of protein; while 3.27 lb of protein are contained in the horny gluten in 100 lb of the high-protein corn. Again, in 100 lb of the low-protein corn the horny starch contains only 3.02 lb of protein; while 4.93 lb of protein are contained in the horny starch in 100 lb of the high-protein corn.

On the other hand, in 100 lb of the low-protein corn the crown starch and tip starch contain 1.53 and .84 lb of protein, respectively; while 1.20

Table 4. Physical and chemical distribution of 100 g (or 100 lb) of corn.

Names of parts	Physical distribution (grams or pounds)	Chemical distribution			
		Protein (grams or pounds)	Oil (grams or pounds)	Ash (grams or pounds)	Carbohydrates grams or pounds)
Ear No. 1 (low in protein)					
Tip caps	1.20	.09	.01	.01	1.09
Hulls	5.47	.27	.05	.04	5.10
Horny gluten	11.61	2.23	.46	.11	8.81
Horny starch	37.15	3.02	.06	.07	34.01
Crown starch	21.26	1.53	.04	.07	19.62
Tip starch	13.71	.84	.04	.04	12.79
Germs	9.59	1.91	3.50	1.01	3.17
Total	99.99	9.89	4.16	1.35	84.59
Whole corn	....	9.28	4.20	1.41	85.11
Ear No. 2 (medium in protein)					
Tip caps	1.46	.13	.03	.02	1.28
Hulls	5.93	.23	.05	.05	5.60
Horny gluten	8.51	1.89	.59	.15	5.88
Horny starch	47.08	4.80	.11	.11	42.05
Crown starch	17.01	1.35	.03	.04	15.59
Tip starch	8.48	.65	.03	.03	7.77
Germs	11.53	2.28	4.02	1.14	4.09
Total	100.00	11.33	4.86	1.54	82.26
Whole corn	....	10.95	4.33	1.55	83.17
Ear No. 3 (high in protein)					
Tip caps	1.62	.08	.03	.03	1.48
Hulls	6.09	.23	.05	.07	5.74
Horny gluten	13.32	3.27	.61	.23	9.20
Horny starch	44.89	4.93	.10	.09	39.76
Crown starch	13.88	1.20	.07	.05	12.56
Tip starch	8.28	.60	.11	.05	7.51
Germs	11.93	2.33	4.02	1.19	4.38
Total	100.01	12.64	4.99	1.71	80.63
Whole corn	......	12.85	5.36	1.67	80.12

Table 5. Pounds of protein in 100 pounds of corn.

Names of parts	Low-protein corn	Medium-protein corn	High-protein corn
In tip caps	.09	.13	.08
In hulls	.27	.23	.23
In horny part	5.25	6.69	8.20
In white starch	2.37	2.00	1.80
In germs	1.91	2.28	2.33
Total	9.89	11.33	12.64

and .60 are the respective amounts contained in the corresponding parts of the high-protein corn.

If we add together the horny parts and then add together the crown starch and tip starch, as is done in the practical selection of seed corn by mechanical examination, we obtain the results shown in Table 5.

It will be observed that the increase in protein in high-protein corn over that in low-protein corn occurs almost entirely in the horny part of the corn kernel. As indicated in previous bulletins, there is also a slight increase in protein in the germ, although this is quite insignificant as compared with the increase in the horny part. In passing from low-protein corn to high-protein corn, there is an appreciable decrease in the amount of protein contained in the white starch. Of course, this is due to the marked decrease in the actual amount of white starch in high-protein corn. Indeed, this decrease in the quantity of white starch is even more marked than would appear from Table 5, because the white starch in the high-protein corn is actually richer in protein than is that in low-protein corn, as would be expected and as is shown in Table 1.

The data given in Table 5 strongly confirm the results which we have already obtained in practical experience in corn breeding. For example, we have been breeding both high-protein corn and low-protein corn for the past 7 years. In the high-protein corn we find that the proportion of horny part has increased very markedly, while the white starchy part has markedly decreased. In the low-protein corn the opposite is true, the horny part having decreased and the white starchy part having markedly increased in proportion.

By computation from the data given in Table 4, we have constructed Table 6, which shows the percentage distribution of the different chemical constituents among the several physical parts of the corn kernel.

It will be seen that, as an average, about 22% of the total protein is contained in the horny gluten, nearly 40% in the horny starch, and nearly 20% in the germ. Thus these three parts contain about 80% of the total protein in the kernel.

The germ contains from 80 to 84% of the oil, while all other parts combined contain only 15 to 20% of the total oil in the kernel. Based upon this fact is the method for selecting high-oil or low-oil seed corn by mechanical examination, the ears whose kernels show a large proportion of germ being high-oil corn and those with small germs low-oil corn (see Fig. 4).

About 12% of the total oil is contained in the horny gluten, leaving only about 5% of the oil distributed among the remaining five physical parts, and, as stated above, more or less of this amount is undoubtedly absorbed from the contiguous germ or horny gluten.

It will be noted that the ash is closely associated with the oil, nearly 75% of the total ash being contained in the germ and about 10% in the horny gluten, as an average.

Table 6. Percentage distribution of chemical constituents among physical parts.

Ear No. 1 (low in protein)

Names of parts	Percent of total protein	Percent of total oil	Percent of total ash	Percent of total carbo-hydrates
In tip caps	.89	.33	.81	1.29
In hull	2.75	1.21	3.34	6.03
In horny gluten	22.56	11.13	7.96	10.41
In horny starch	30.51	1.43	4.98	40.22
In crown starch	15.52	.97	5.07	23.18
In tip starch	8.46	.95	2.96	15.12
In germs	19.31	83.99	74.87	3.75
Total	100.00	100.01	99.99	100.00
Ear No. 2 (medium in protein)				
In tip caps	1.14	.69	1.06	1.56
In hull	2.07	1.08	3.06	6.80
In horny gluten	16.67	12.21	9.56	7.15
In horny starch	42.36	2.32	7.38	51.12
In crown starch	11.88	.59	2.67	18.96
In tip starch	5.75	.68	1.72	9.45
In germs	20.14	82.43	74.55	4.97
Total	100.01	100.00	100.00	100.01
Ear No. 3 (high in protein)				
In tip caps	.59	.65	1.76	1.84
In hull	1.85	.93	3.90	7.12
In horny gluten	25.88	12.29	13.49	11.41
In horny starch	39.00	1.98	5.49	49.31
In crown starch	9.45	1.44	2.99	15.58
In tip starch	4.77	2.25	2.89	9.32
In germs	18.45	80.46	69.46	5.43
Total	99.99	100.00	99.98	100.01

Table 7 shows, for direct comparison, the percentage distribution of the protein among the different physical parts in each ear, the two horny parts, and also the two white starchy parts, being combined, as in Table 5.

Table 7 illustrates very plainly the fact that, as we pass from low-protein corn to high-protein corn, the protein decreases in the white starchy part and increases in the horny part; in other words, in breeding corn for high protein we decrease the white starchy part, which is comparatively poor in protein, and increase the horny part, which averages very much richer in protein, the horny starch containing from 2 to 3% more protein than the white starch, and the horny gluten being richer in protein than any other part of the kernel. As a rule, in breeding for high protein there is also a slight increase in the proportion of germ, which, being rich in protein, adds somewhat to the increase in protein.

Table 7. Distribution of 100 g (or 100 lb) of protein among the physical parts as observed in mechanical examination.

Names of parts	Low-protein corn	Medium-protein corn	High-protein corn
In tip caps	.89	1.14	.59
In hulls	2.75	2.07	1.85
In horny part	53.07	59.03	64.88
In white starch	23.98	17.63	14.22
In germs	19.31	20.14	18.45
Total	100.00	100.01	99.99

MECHANICAL METHODS OF SELECTING SEED CORN FOR IMPROVEMENT IN COMPOSITION

As has already been shown in our Bulletin No. 82, "Methods of Corn Breeding," we have found it entirely feasible and practical to select seed corn of higher protein content by a simple mechanical examination of a few kernels from each ear. With some care any farmer or corn grower can learn to pick out high-protein seed corn by dissecting and examining a few kernels from each ear (by means of a pocketknife), selecting for high-protein seed the ears whose kernels show a large proportion of horny part, and rejecting those showing a small proportion of horny part (see Figs. 1 to 3).

This method is already in use by practical corn breeders, and with a very satisfactory degree of success. For example, in selecting seed corn by this method, Mr. Ralph Allen, of Tazewell County, obtained seed ears for the year 1902 which were 1.46% higher in protein than the rejected ears from the same lot, and for this season (1903) his selected seed ears contain 1.58% more protein than the ears which he has rejected. In other words, his selected seed corn is richer by 1.58 lb of protein per 100 lb of corn than that rejected.

The method proposed some years ago by Professor Willard, Director of the Kansas Agricultural Experiment Station, of picking out high-protein seed by simply selecting for large germs, enables one, as a rule, to make some gain in protein, but the gain is very much greater when the proportion of horny part is considered. In fact, from our own experience we find that the selection for a large proportion of horny part is a very much more trustworthy index than the size of the germ in securing high-protein seed, and we often find corn with large germs which is actually low in protein because of a small percentage of protein in the remainder of the kernel. The fact that only 20% of the total protein of the kernel is obtained in the germ (as shown in Table 7) is evidence of the uncertainty of obtaining high-protein seed corn, and of the improbability of making any very considerable gain in protein, by this method of selection. This difficulty was well understood by Professor Willard, as will be seen in the following quotation from the Kansas Experiment Station Bulletin No. 107, page 63.

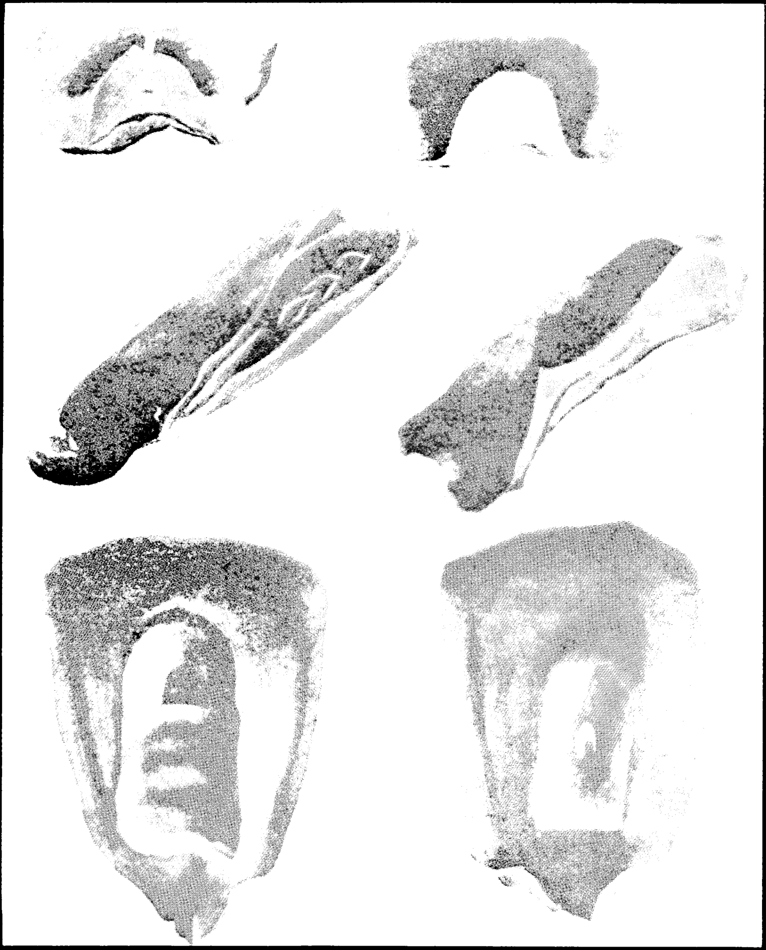

Figure 3. Left: High-protein kernels (much horny part; little white starch). Right: Low-protein kernels (little horny part; much white starch).

"There are undoubtedly great differences in the protein content of the part of the kernel exclusive of the germ, and it is conceivable and not improbable that a large germ, though in itself tending to produce high protein content might be overcome by the low protein of the remainder of the kernel." (Protein is substituted for nitrogen in this quotation.)

Of course, if one picks out corn with large germs and at the same time, either consciously or unconsciously, selects those ears whose kernels contain a large proportion of horny part, he may make considerable gain in protein, but in such case the gain should not be attributed solely to the large germs.

The method of selecting seed corn for high oil content on the basis of large germs (Fig. 4) is certainly well founded, because of the fact that more than 80% of the total oil of the kernel is contained in the germ.

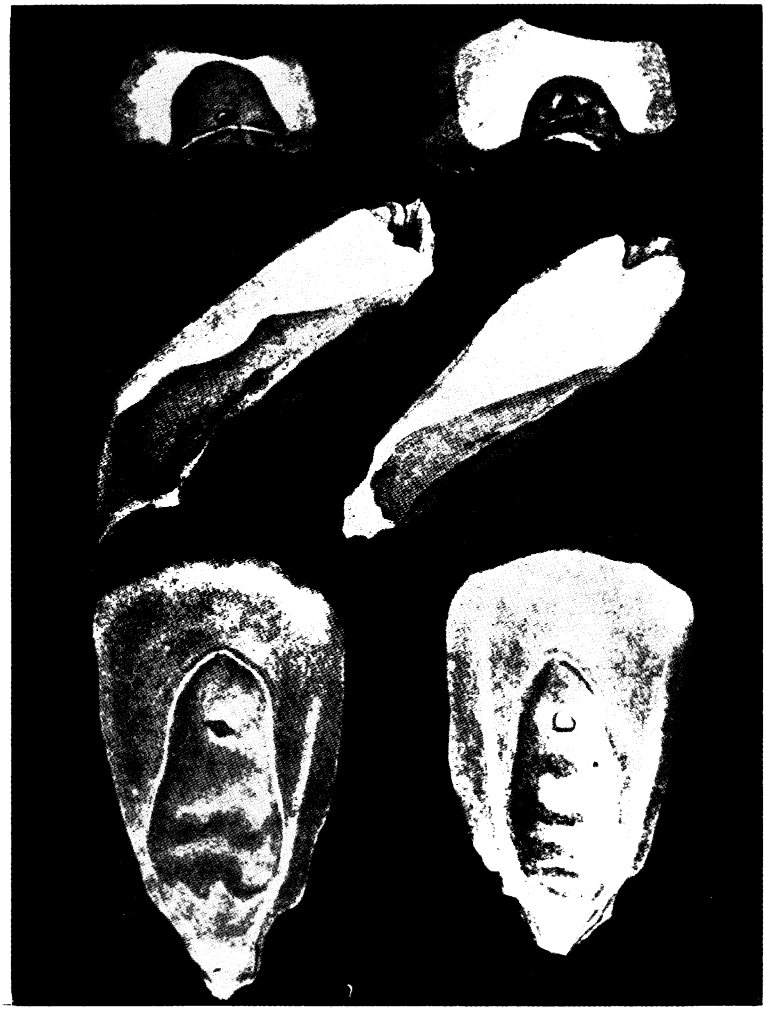

Figure 4. Left: High-oil kernels (large germs). Right: Low-oil kernels (small germs).

THE CORRELATION OF SOME PHYSICAL PARTS AND CHEMICAL CONSTITUENTS OF THE CORN KERNEL

As was clearly shown in our Bulletin No. 55, there is usually a marked correlation between the percentage of germ and the percentage of oil in the corn kernel, as will be seen from the following summary of 100 separate determinations reported in that bulletin.

While there is, of course, some variability, due in part to the different percentages of oil in germs from different kernels (especially in kernels from

Table 8. Correlation between germ and oil in corn kernels.

Number of determinations	Small-germ corn		Large-germ corn	
	Germ, percent	Oil, percent	Germ, percent	Oil, percent
10	**9.10**	3.58	**14.11**	6.49
10	**8.56**	3.22	**12.40**	6.71
10	**8.28**	3.64	**12.01**	6.08
10	**8.73**	3.32	**13.30**	5.82
10	**9.82**	4.30	**11.06**	5.21
General average	**8.90**	3.61	**12.57**	6.06

different ears) and in part to the different percentages of oil in the horny gluten (and to some extent in other parts) from different ears (see Table 8), nevertheless it will be observed that there is a marked correlation between the percentage of germ and the percentage of oil in the corn kernel. In other words, the percentage of oil varies with the percentage of germ. It will be observed, however, that a high percentage of germ is accompanied by a still higher proportionate percentage of oil, indicating that increased proportion of germ in the kernel is due to an increase in the quantity of oil more largely than of the remainder of the germ.

CORRELATION BETWEEN OIL AND PROTEIN

That there is a marked correlation between the percentages of germ and oil in the corn kernel is certainly well established; consequently, if the proportion of germ were a reliable index to the relative protein content of the kernel, there would, of course, be some marked correlation between the percentages of oil and protein in corn.

In Table 1 of Bulletin No. 55 are recorded the proximate analyses of 163 different ears of corn. Table 9 is derived from that data, and shows the percentages of oil and protein contained in the ten ears which are lowest in oil and in the ten ears which are highest in oil, the analyses being arranged in the descending order for protein.

The correlation between oil and protein is certainly very slight. In the low-oil corn the oil varies from only 3.84 to 4.08%, while the protein varies from 9.08 to 12.96%. In the high-oil corn the oil varies only from 5.46 to 6.02, while the protein varies from 9.58 to 13.87%, making a difference of 4.29% protein between these extreme ears, while the difference in oil is only .09% between the same ears. The average percentages of oil in these two lots of corn vary from 3.99 to 5.64%, while the corresponding averages for protein are 11.02 and 11.19, thus showing very little correlation between the percentages of oil and protein in the corn kernel.

Table 10 is also derived from Table 1 of Bulletin No. 55, and is similar to Table 9, except that the analyses of the ten ears lowest in protein and of the ten ears highest in protein are chosen. This shows the reverse correlation;

Table 9. Correlation between oil and protein in the corn kernel.

Low-oil corn		High-oil corn	
Oil, percent	Protein, percent	Oil, percent	Protein, percent
3.97	12.96	5.72	13.87
3.99	12.28	5.51	13.10
4.03	11.71	5.61	12.09
4.07	11.49	5.75	11.18
3.84	11.29	5.65	10.82
4.08	11.29	5.51	10.49
3.94	10.97	5.46	10.32
4.01	9.68	5.51	10.23
3.95	9.44	6.02	10.18
4.05	9.08	5.63	9.58
Average 3.99	11.02	5.64	11.19

Table 10. Correlation between protein and oil in the corn kernel.

Low-protein corn		High-protein corn	
Protein, percent	Oil, percent	Protein, percent	Oil, percent
9.31	4.96	13.87	5.72
8.40	4.91	13.10	5.51
8.38	4.88	12.68	5.29
9.31	4.82	12.81	5.21
8.25	4.81	12.63	5.15
9.22	4.60	13.06	4.93
9.15	4.55	12.57	4.82
9.30	4.38	12.79	4.25
9.12	4.10	12.76	4.10
9.08	4.05	12.96	3.97
Average 8.95	4.61	12.92	4.90

that is, between protein and oil. Of course, the results should be practically the same as shown in Table 9, the chief value of Table 10 being that it uses almost an entirely different set of analyses, and consequently gives a duplicate illustration of the lack of correlation between these two constituents.

It will be observed that although the averages for protein vary from 8.95 to 12.92%, the averages for oil vary only from 4.61 to 4.90%. These averages indicate only a slight correlation between protein and oil, and the analyses of the individual ears show that even this correlation is by no means constant.

In connection with some investigations relative to heredity, Professor Frank Smith of this university has prepared correlation tables (see Tables 11 and 12) from the 163 analyses of individual ears of corn recorded in Table 1 of Bulletin No. 55. Table 11 shows the correlation between protein and carbohydrates, the entire 163 ears of corn being grouped according to the percentages of protein and carbohydrates which they contain. This table well illustrates what is meant by a high degree of correlation. With an increasing protein percentage there is a decreasing percentage of carbohydrates, and

Table 11. **One hundred sixty-three ears of corn grouped according to percentages of protein and carbohydrates.**

Carbo-hydrates, percent	Protein, percent													Total No. of ears
	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	
79													1	1
79.5														
80											1			1
80.5										1	2			3
81									1	3				4
81.5							1		2	7	3			13
82							2	5	13					20
82.5					1	3	7	12	3	1				27
83					2	7	16	7						32
83.5				2	7	9	4	1						23
84				3	7	5	1							16
84.5			1	8	2									11
85			1	5										6
85.5	1	2	2	1										6
Total No. of ears	1	2	4	19	19	24	31	25	19	12	6		1	163

The correlation of high protein with low carbohydrates and *vice versa* equals 90.05 percent.

there are no marked exceptions to this rule. Note, for example, that there is one ear containing 14% of protein, but that this ear contains only 79% of carbohydrates, being the highest in protein and also the lowest in carbohydrates of the 163 ears. There are 19 ears containing only 12% of protein, but none of these 19 ears contains less than 81% of carbohydrates; 13 of them contain 82% of carbohydrates, while 5 others are within .5% of that amount. One ear contains only 8% of protein, but this ear contains 85.5% of carbohydrates, being both the lowest in protein and the highest in carbohydrates of the 163 ears. It will be observed that of the 163 ears, several groups fall in the squares representing low protein (8 to 10%) and high carbohydrates (84 to 85.5%); also that many ears fall in the squares representing high protein (12 to 14%) and low carbohydrates (80 to 82%); so that, including the ears with medium content of protein and carbohydrates, we find that the group numbers of the 163 ears fall almost in a straight line extending from the lower left-hand corner to the upper right-hand corner of the table. It will be seen that no ears whatever fall in the squares representing high protein and high carbohydrates or in those representing low protein and low carbohydrates. By mathematical computation Professor Smith has found that we have in this table 90.05% of a perfect correlation.

Table 12. One hundred sixty-three ears of corn grouped according to percentages of protein and oil.

Oil, Percent	Protein, percent													Total No. of ears
	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	
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 No. of ears	1	2	4	19	19	24	31	25	19	12	6		1	163

The correlation of high protein with high oil and of low protein with low oil equals 3.81 percent.

Table 12 shows the degree of correlation which exists between oil and protein. It will be seen that there is no such grouping as in Table 11. In other words, there is no marked correlation between the oil and the protein in corn. Some ears are rich in one of these constituents and poor in the other; other ears are rich in both, and still others are poor in both, so that the grouping of the numbers of ears according to the percentages of oil and protein which they contain resembles a circle much more nearly than a straight line. By computation it is found that there is a slight tendency for the protein to increase with increasing oil content, but the degree of correlation amounts to only 3.81% of a perfect correlation.

All of the above data tend to prove that as the percentage of protein increases in corn the starch decreases, while the oil remains almost unchanged; and that we may increase or decrease the percentage of oil or of germ in corn without markedly affecting the percentage of protein. This was the conclusion drawn when the above-mentioned 163 ears of corn were analyzed more than 6 years ago. The different strains of corn which we have finally produced in our regular corn-breeding work furnish us excellent material for ascertaining what effect is produced upon the oil content of corn by breeding for a higher or lower protein content; and, vice versa, what effect is produced upon the protein content by breeding for a higher or lower oil content.

In 1900 we planted 10 field rows (called the "mixed plot") with two

Table 13. Oil and protein in corn harvested from the "mixed-oil" plot in 1900.

Row No.	Low-oil side		High-oil side	
	Oil, percent	Protein, percent	Oil, percent	Protein, percent
1	**3.93**	10.07	**5.61**	10.06
2	**3.78**	9.26	**5.74**	9.05
3	**3.73**	10.21	**5.88**	9.12
4	**3.75**	8.47	**5.99**	9.65
5	**3.89**	9.39	**5.71**	10.08
6	**3.80**	9.77	**5.91**	10.23
7	**3.60**	9.80	**5.60**	9.91
8	**3.58**	9.65	**5.84**	10.32
9	**4.22**	9.18	**5.68**	9.15
10	**3.27**	9.26	**5.82**	9.32
Average......	**3.81**	9.51	**5.78**	9.69

kinds of corn in every row, one kind having been bred for 4 years for high oil content, the other (originally from the same variety and stock) having been bred during the same 4 years for low oil content. These two kinds of seed were planted in every row and in fact in every hill, the low-oil kernels and the high-oil kernels in the same hill—just far enough apart so that the identity of the individual plants could be known as they grew during the season. The corn from each of the ten rows was harvested in two lots, one lot being corn from high-oil seed and the other lot being from low-oil seed. The two lots from each row were kept separate, the one being labeled as corn from the "high-oil side" of the row and the other from the "low-oil side."

The percentages of oil and of protein contained in these different lots of corn are shown in Table 13.

These data are considered very reliable, both kinds of corn having been grown during the same season and in exactly the same soil and each individual sample whose composition is shown in Table 13 being a composite sample representing many ears. The average difference in oil content between the high-oil side and the low-oil side is 1.97% of oil, while the average difference in protein is .18%. Considering that the percentage of protein in the corn is twice as large as the percentage of oil, it will be seen that there is less than 5% of a perfect correlation between the oil and protein.

COMPOSITION OF PEDIGREED CORN

In order that it might be shown with even more absolute certainty which physical part of the corn kernel should be increased in order to increase the protein content, or the oil content, etc., 40 ears of corn were selected from the 1902-crop from our oldest breeding plots, 10 ears being taken from each of 4 different strains, namely:

"Illinois" High-Protein Corn
"Illinois" Low-Protein Corn
"Illinois" High-Oil Corn
"Illinois" Low-Oil Corn

each of which represents the seventh generation of pedigreed corn, bred as indicated by the name. Twenty-five average kernels were taken from each of these 40 ears, the germs separated from the remainder of the kernel and both parts (that is, germs and endosperms[3]) analyzed separately, for each ear, another sample of the corn from each ear also being analyzed to show the composition of the whole corn. The tabular statements show the results obtained.

By referring to Tables 14 and 15 it will be seen that the protein content of the low-protein ears varies from 6.36 to 7.09%, with an average of 6.71%, while the protein content of the high-protein ears varies from 13.98 to 15.01, with an average of 14.44%. The average oil content of the low-protein corn is 4.21% and of the high-protein ears 4.93%. The general averages indicate a slight correlation between oil and protein; however, there are several of the high-protein ears which contain less oil than some of the low-protein ears, thus showing that such correlation is not constant.

Table 16 shows the percentages of protein and of germ in both the low-protein and high-protein ears.

Here again we see a slight correlation between the average percentage of protein and that of germ in the corn kernel, although there are noteworthy discrepancies. Thus we have an ear containing 6.37% of protein and 9.53% of germ, while another ear contains 14.74% of protein and 9.51% of germ.

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[3] As here used, the term "endosperm" includes all parts of the kernel except the germ.

Table 14. Chemical composition of ten ears of "Illinois" low-protein corn.

Ear No.	Protein, percent	Oil, percent	Ash, percent	Carbohydrates, percent
4276	6.98	4.69	1.43	86.90
4281	6.60	4.21	1.33	87.86
4286	6.87	4.16	1.19	87.78
4287	6.37	3.90	1.40	88.33
4295	6.46	4.14	1.15	88.25
4313	7.01	4.13	1.27	87.59
4321	7.09	3.84	1.46	87.61
4328	6.36	4.05	1.43	88.16
4346	6.89	4.33	1.45	87.33
4368	6.48	4.67	1.56	87.29
Average.......	6.71	4.21	1.37	87.71

Table 15. Chemical composition of ten ears of "Illinois" high-protein corn.

Ear No.	Protein, percent	Oil, percent	Ash, percent	Carbohydrates, percent
4174	14.70	5.87	1.48	77.95
4189	14.74	4.46	1.70	79.10
4202	14.21	4.54	1.50	79.75
4212	14.61	4.57	1.66	79.16
4218	14.37	4.61	1.63	79.39
4227	14.03	6.26	1.54	79.17
4242	14.28	5.33	1.51	78.88
4244	14.49	4.71	1.57	79.23
4253	15.01	5.01	1.38	78.60
4265	13.98	4.96	1.67	79.39
Average	14.44	4.93	1.56	79.06

Table 16. Protein and germ in low-protein and high-protein corn.

Low-protein corn			High-protein corn		
Ear No.	Protein, percent	Germ, percent	Ear No.	Protein, percent	Germ, percent
4276	6.98	9.52	4174	14.70	13.31
4281	6.60	8.81	4189	14.74	9.51
4286	6.87	8.42	4202	14.21	11.44
4287	6.37	9.53	4212	14.61	10.92
4295	6.46	7.95	4218	14.37	11.64
4313	7.01	8.98	4227	14.03	11.15
4321	7.09	10.30	4242	14.28	13.21
4328	6.36	8.88	4244	14.49	11.22
4346	6.89	10.14	4253	15.01	9.82
4368	6.48	10.79	4265	13.98	12.14
Average....	6.71	9.33	Average....	14.44	11.44

In other words, the two ears contain practically the same percentage of germ, although one of them contains more than twice as much protein as the other. One of the lowest-protein ears (6.48%) contains 10.79% of germ, while the highest protein ear (15.01%) contains only 9.82% of germ.

Attention is called to the fact that in selecting seed corn by chemical analysis for high protein there is a tendency to increase not only the horny starchy part (which contains more total protein than any other part of the corn kernel), but also to increase both the horny gluten and the germ, both of which, although small in amount, are rich in protein; and consequently there is a slight tendency for the oil to be increased, not only in the germ, but also in the horny gluten (aleurone layer), which it will be remembered is also quite rich in oil. This is the evident explanation as to why there is a slightly higher degree of correlation between oil and protein in our pedigreed strains of corn than there is in ordinary corn which has not been so bred.

Tables 17 and 18 show the percentage composition of the low-oil and high-oil ears.

The average for the low-oil corn is 2.52% of oil and 9.98% of protein, while the high-oil contains 7% of oil and 11.31% of protein. In other words, the high-oil corn contains almost three times as much oil as the low-oil corn but is less than one-seventh richer in protein, showing only slight correlation between oil and protein, and with several ears no correlation whatever exists. For example, we have one ear with 10.89% of protein and 2.40% of oil, and another ear with 10.79% of protein and 7.01% of oil, the protein being practically equal, while the one ear contains nearly three times as much oil as the other. Again, the low-oil ear No. 4555 (2.65% of oil) contains 11.92% of protein, or .61% more than the average of all of the high-oil ears.

Table 19, giving the percentages of oil and germ in the low-oil and high-oil corn, shows a very marked correlation between oil and germ. The 10 low-oil ears contain from 2.12 to 2.68% of oil (average 2.52) and from 7.06 to 8.47% of germ (average 7.74), while the 10 high-oil ears contain from 6.74

Table 17. Chemical composition of ten ears of "Illinois" low-oil corn.

Ear No.	Protein, percent	Oil, percent	Ash, percent	Carbohydrates, percent
4474	9.40	**2.68**	1.45	86.47
4486	9.16	**2.65**	1.64	86.55
4491	9.49	**2.60**	1.29	86.62
4495	9.57	**2.59**	1.41	86.43
4509	8.96	**2.53**	1.36	87.15
4512	10.64	**2.45**	1.46	85.45
4521	9.97	**2.12**	1.42	86.49
4537	10.89	**2.40**	1.54	85.17
4548	9.77	**2.54**	1.36	86.33
4555	11.92	**2.65**	1.42	84.01
Average......	9.98	**2.52**	1.44	86.07

Table 18. Chemical composition of ten ears of "Illinois" high-oil corn.

Ear No.	Protein, percent	Oil, percent	Ash, percent	Carbohydrates, percent
4374	11.26	**7.10**	1.64	80.00
4411	10.79	**7.01**	1.40	80.80
4412	9.58	**6.87**	1.58	81.97
4417	10.33	**7.01**	1.66	81.00
4421	12.55	**7.02**	1.53	78.90
4423	11.66	**6.95**	1.56	79.83
4436	11.47	**7.17**	1.59	79.77
4441	12.94	**7.37**	1.57	78.12
4448	11.75	**6.78**	1.52	79.95
4462	10.76	**6.74**	1.48	81.02
Average	11.31	**7.00**	1.55	80.14

Table 19. Oil and germ in low-oil and high-oil corn.

Low-oil corn			High-oil corn		
Ear No.	Oil, percent	Germ, percent	Ear No.	Oil, percent	Germ, percent
4474	**2.68**	8.05	4374	**7.10**	12.90
4486	**2.65**	8.13	4411	**7.01**	12.73
4491	**2.60**	7.92	4412	**6.87**	13.73
4495	**2.59**	7.39	4417	**7.01**	14.50
4509	**2.53**	7.06	4421	**7.02**	14.65
4512	**2.45**	7.89	4423	**6.95**	13.83
4521	**2.12**	7.13	4436	**7.17**	14.10
4537	**2.40**	7.57	4441	**7.37**	14.53
4548	**2.54**	7.83	4448	**6.78**	14.35
4555	**2.65**	8.47	4462	**6.74**	13.03
Average...	**2.52**	7.74	Average...	**7.00**	13.84

to 7.37% of oil (average 7), and from 12.73 to 14.65% of germ (average 13.84). There is no overlapping, and the correlation is very distinct. Every low-oil ear contains a small percentage of germ and every high-oil ear a high percentage of germ. Attention is called to the fact that the high-oil corn is even richer in oil than would be indicated by the high percentage of germ as compared with the percentage of oil and germ in the low-oil corn, indicating that the breeding for high-oil has not only increased the oil by increasing the percentage of germ (which contains most of the oil), but that the percentage of oil in the germs itself has increased. (Of course, there is also an increase in the percentage of oil in the horny glutenous part.) Similarly, the percentage of oil has decreased even more rapidly than the percentage of germ in the low-oil corn. These results are very apparent in the data shown in Table 20.

EFFECT OF BREEDING ON COMPOSITION OF GERMS AND ENDOSPERMS

As already explained, 10 ears were selected from each of the 4 different strains of corn (low-protein, high-protein, low-oil, and high-oil), and 25 kernels were taken from each of these 40 ears, the germ being separated from the remainder of the kernel, which we call the endosperm. After the percentage of germ was determined from each individual ear, the germs from each lot of 10 ears were put together to make 2 samples, each sample representing 5 ears. The endosperms were likewise put together, so that we have duplicate samples of both germs and endosperms for each of the four different strains. These samples were analyzed chemically and the results are given in Table 20.

Table 20. Chemical composition of germs and endosperms from low-protein and high-protein corn and from low-oil and high-oil corn.

Kind of corn	Part of kernel	Protein, percent	Oil, percent	Ash, percent	Carbohydrates, percent
Low-protein......	Germs	**18.05** **17.96**	33.59 34.60	10.19 10.16	38.17 37.28
High-protein.....	Germs	**20.85** **21.65**	34.99 36.02	10.12 10.07	34.04 32.26
Low-oil..........	Germs	21.70 21.71	**25.01** **24.62**	13.13 13.36	40.16 40.31
High-oil..........	Germs	17.55 17.84	**41.76** **41.75**	8.75 8.81	31.94 31.60
Low-protein......	Endosperms	**5.69** **5.68**	.83 .91	.43 .43	93.05 92.98
High-protein.....	Endosperms	**13.67** **13.92**	.76 .72	.36 .41	85.21 84.95
Low-oil..........	Endosperms	9.13 9.14	**.52** **.51**	.47 .43	89.88 89.92
High-oil..........	Endosperms	10.62 10.10	**1.07** **1.24**	.36 .39	87.95 88.27

These results show in a very striking manner the effect of breeding in changing the composition of the different physical parts of the kernel. Thus the germs from the low-oil corn contain about 25% of oil, while those from the high-oil corn contain nearly 42% of oil. As stated above, breeding to change the oil content not only changes the percentage of germ, but it also changes the percentage of oil in the germ. It should also be noted that endosperms from the high-oil corn contain more than twice as much oil as those from the low-oil corn, although the percentage of oil in the endosperm is very small even in the high-oil corn, and this oil is largely contained in the horny gluten.

Perhaps the most marked and valuable results are shown in the percentages of protein contained in the endosperms from low-protein and high-protein corn; the endosperms from the low-protein corn contain less than 6% of protein, while those from the high-protein corn contain nearly 14% of protein. These results, in connection with others which we have given, would seem to prove very conclusively that to select high-protein seed corn by mechanical examination we should select principally for a large proportion of the more nitrogenous part of the endosperm; that is, the horny part. To select only for large germs will have but a slight effect upon the protein content of the corn, although it will produce a rapid and marked increase in the oil content.

Referring again to Table 20, it will be seen that the endosperms from the high-oil corn contain about 1% more protein than those from the low-oil corn. On the other hand, the germs from high-oil corn contain less protein (17.7%) than those from low-oil corn (21.7%), the difference being 4% protein in favor of the low-oil corn.

These results were to be expected even from a study of the analyses of the 163 ears reported in Bulletin No. 55, in 1899, which showed that large germs were naturally even richer in oil than the size of the germs would indicate, and that there is but very slight correlation between oil and protein, the increased oil tending to decrease the percentage, though not the actual amount of protein in the germ. It will be seen from Tables 19 and 20 that the high-oil corn contains nearly twice as much germ as the low-oil corn, and that the germs from high-oil corn are more than one and one-half times richer in oil than the germs from the low-oil corn; but that, although the high-oil germs contain a larger total amount of protein, because of their increased size, they are really considerably poorer in percentage of protein than the low-oil germs.

It is perhaps worth while to consider the evident fact that, even if the protein should increase in the germ in the same proportion as the oil (which is not the case), we should need to increase the oil 2 lb for every 1 lb increase in protein obtained, if we depend upon the method of picking our high-protein seed corn by selecting for large germs. In other words, to increase the protein in corn from 10% to 15% by this method would require the oil in the corn to be increased from 5% to 15%.

Although we do not assume to say what should be the percentage of oil in corn for feeding purposes, we do take the liberty of raising the question whether the popular opinion that the oil in corn should be increased for feeding purposes may not be erroneous. Certainly the investigations of Lehmann in Germany and of Shutt in Canada have indicated very strongly that corn is already too rich in oil to be suitable as a foodstuff for bacon hogs. It may also be called to mind that some other excellent foodstuffs, such as oats, bran, barley, red clover, and alfalfa, contain less than half as much oil as is already contained in ordinary corn.

Attention is called to the fact, that although the physical parts of the corn kernel which contain nearly all of the oil (namely the germ and the horny gluten) also contain most of the ash, yet a high percentage of ash in the germs is associated with a low percentage of oil, and vice versa, indicating that the ash content of the germ (which includes the major part of the ash of the entire kernel) bears a more constant relation to the oil-free material in the germ than to the whole germ. By computation we find that the oil-free germs contain the percentages of ash given in Table 21 (assuming the oil to contain no ash, which is approximately correct).[4]

Breeding for high or low protein produces no marked effect upon the ash content or the oil content of either the germs or the endosperms, and only slightly influences the protein content of the germs. (The low-protein germs contain about 18% of protein and the high-protein germs about 21%.) The results show that such breeding produces exceedingly marked effects upon the protein content of the endosperms, the low-protein endosperms containing less than 6% and the high-protein endosperms nearly 14% of protein. In this connection it is well to remember that the corn kernel usually contains only about 11% of germ while the endosperm amounts to about 89% of the kernel. The significance of this becomes more readily apparent by an examination of Table 22, which shows where the protein actually exists in 100 lb of corn.

We thus find as a result of corn breeding that in the seventh generation we have a maximum difference of only .75 lb of protein in the germs from 100 lb of low-protein and high-protein corn, while in the endosperms from

Table 21. Percentage of ash in germs.

	In fresh germs	In oil-free germs
From low-protein corn	10.19	15.34
	10.16	15.54
From high-protein corn	10.12	15.57
	10.07	15.74
From low-oil corn	13.13	17.51
	13.36	17.72
From high-oil corn	8.75	15.02
	8.81	15.12

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[4] Actual determinations of the ash in corn oil have shown that the oil contains only 2% of ash.

these two kinds of corn we have a difference of 7.06 lb of protein in 100 lb of corn. In other words, in changing the protein content of corn the effect produced in the endosperms amounts to almost ten times the effect produced in the germs.

THE COMPOSITION OF HOMINY MILL PRODUCTS

Besides the investigations which we have carried on along this particular line in connection with our work of corn breeding, we have also made some study of factory products, especially the products from hominy mills, which make use of immense quantities of corn.

In the regular process of milling corn, a very large number of separations are made and several distinctly different final products are obtained, some of which are composed almost entirely of certain distinct physical parts of the corn kernel. The following is a brief and very general description of the usual process of corn milling:

The whole corn is somewhat softened by steaming and is then run through a hulling machine, which not only removes the hull but loosens the germ and breaks off the horny gluten and more or less white starch. The dust, or pulverized material, coming from the hulling machine consists largely of white starch and horny gluten. The hulls and germs are each separated, but not in very pure condition, leaving what is termed hominy, which consists chiefly of the horny starchy part of the kernel with more or less adhering white starch.

The product which is known as grits is made from the hominy and consists of the horny starchy part separated in very pure form. In making grits the coarse hominy is run through a grinding machine and reduced to a coarse powder, which may be termed coarse grits, much of the adhering white starch being rubbed off from the horny starch in this process. The coarse grits are then run through one or two more grinding machines, until the horny starch is reduced to a rather fine powder, which may be termed fine grits. This material consists of the horny starch in very pure condition. After each grinding, the fine dust, consisting largely of the white starch, is separated from the grits and goes into the product known as corn flour.

In addition to the corn flour thus regularly separated and handled in considerable quantities, there is constantly produced a small amount of what is termed "break" flour. This is an exceedingly fine dust also produced in

Table 22. Protein in 100 pounds of corn.

Names of parts	Low-protein corn			High-protein corn			Difference
	Percent of corn	Percent of protein	Pounds of protein	Percent of corn	Percent of protein	Pounds of protein	Pounds of protein
In germs....	9.33	18.01	**1.68**	11.44	21.25	**2.43**	.75
In endosperms....	90.67	5.69	**5.16**	88.56	13.80	**12.22**	7.06

Table 23. Composition of parts of the corn kernel separated by hominy mill and by hand.

Names of parts	Methods of separation	Protein, percent	Oil, percent	Ash, percent	Carbohydrates, percent
Hulls....................	By mill	6.85	2.94	1.11	89.10
Hulls....................	By hand	4.97	.92	.82	93.29
Horny starch (fine grits)	By mill	8.46	.44	.26	90.84
Horny starch...........	By hand	8.12	.16	.18	91.54
White starch (corn flour*)	By mill	5.91	1.63	.49	91.97
White starch (break flour)	By mill	5.88	2.04	.68	91.40
White starch (from tip)	By hand	6.10	.29	.29	93.31
Germs..................	By mill	15.84	21.26	7.41	55.49
Germs..................	By hand	19.91	36.54	10.48	33.07
Whole corn	Mill sample	9.31	4.20	1.43	85.06
Whole corn.............	Ear No. 1	9.28	4.20	1.41	85.11

*Obtained by direct separation from grits.

the process of breaking the corn particles in the grinding machines which reduce the hominy to grits. The break flour is carried from the machine by an air current through conduits and finally collected. This is another very pure form of the white starch.

Thus, in the regular milling process there are two physical parts of the corn kernel that are separated in very pure form: namely, horny starch (fine grits) and white starch (break flour or corn flour), and two other distinct parts which are separated somewhat less perfectly, the hulls and the germs.

By the courtesy of the manager of the American Hominy Company's Mills at Decatur we were allowed to collect representative samples of these different products for analysis. The composition of these products is given in Table 23, and it will be found interesting to compare these results with the composition of the same products, or parts, which were obtained by exact hand separation, as given in Table 1. For convenience in comparison, Table 23 also shows the composition of these parts as obtained from Ear No. 1 (Table 1), which is very similar to the corn which was being used in the mill at the time the samples were taken. This was fairly representative of the ordinary white corn grown in 1902, nearly all of which was abnormally low in protein, owing to seasonal influences.

In general the composition of these mill separations agrees with the composition of the same parts separated by hand, although in nearly all cases the mill products show more or less contamination or mixture with other parts of the kernel. Thus the mill hulls are noticeably high in protein and oil owing to the presence of some particles of horny gluten and germ; while the mill germs are too low in protein and oil because of the presence of some hulls and tip caps. Furthermore, some oil is lost from the germ and absorbed by other parts in the milling process. The fine grits are almost pure horny starch, except that they contain about twice as much oil as the hand-separated product. This is doubtless due to the fact that some germs are broken or crushed in the hulling machine and the liberated oil is absorbed to some extent by the hominy, chiefly, of course, by the white starch, as indicated by the high oil content of the break flour and the other regularly separated corn flour, although it is evident that a small portion of this liberated oil remains adhering to the fine grits. The white starch contains 5.88 to 5.91% of protein, while the horny starch (fine grits) contains 8.46%, or almost one-half more.

It will be observed that the two samples of whole corn are almost identical in composition. While the corn is fairly representative of much of the white corn grown during the season of 1902, attention is called to the apparent fact that this is not the most suitable corn for the manufacture of hominy and grits. It seems evident that corn containing a higher percentage of the horny starchy part would be more valuable for the hominy mill. The manager of the American Hominy Company's Mills at Decatur has assured the writer that he prefers corn which shall run high in grits (horny starch), but he does not desire that the oil content should be increased; indeed, it would be much better for milling purposes to have the percentage of oil in corn reduced, because of the difficulty of preventing the oil from being absorbed by other products and injuring their quality, the tendency being for the oil to become rancid when exposed to the air. The hominy mills offer some encouragement to farmers to grow corn especially suited to their use.

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3

Ten Generations of Corn Breeding

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INTRODUCTORY NOTE

In 1896 the Illinois Experiment Station began the improvement of corn by varying the composition of the grain through selection and breeding. The results of the first 2 years of these investigations were published in Bulletin No. 55 "Improvement in the Chemical Composition of the Corn Kernel." This same work has been carried on continuously since that time, and although several publications have been issued in the meantime bearing upon different phases of the subject of corn improvement as it has been developed, including Bulletins 82, 87, 100, and 119, there has, however, been no complete report published of the results obtained in the progress of this original line of work described in Bulletin No. 55.

It is the present purpose therefore to present the results which have been obtained, in the first ten generations, in improvement in composition in various directions, namely, for high protein, for low protein, for high oil and for low oil, of the single variety of corn from which these four different strains have been produced, and which is known as "Illinois" corn.

Since the discovery of the possibility of improving corn for special adaptation and the general recognition of its importance, this Station has extended its work to other standard varieties of corn best adapted to different sections of the state, applying the methods and principles worked out in the original experiments with the "Illinois" variety. This work of improving the other standard varieties both for yield and for special adaptation is being carried on largely in co-operation with seed corn breeders of the state. A large amount of data relating to this later work has already accumulated, but it is proposed to reserve this for future publication rather than to attempt to cover in this report all of the corn breeding work now in progress believing that such a division of the subject will allow a clearer presentation.

Inasmuch as the editions of Bulletins 53 and 55 are already exhausted and the demand for the information contained therein is still unsatisfied, it is proposed to make the nature of this report as complete a presentation of the investigation as is possible without making too great repetition of material already published.

For several years Professor Louie H. Smith has been largely responsible for the conduct of these investigations, valuable assistance having also been rendered during recent years by Doctor E. M. East, now agronomist at the Connecticut Experiment Station, Mr. R. W. Stark, now chief chemist for the Cuban Experiment Station, Mr. H. H. Love, and Mr. C. H. Myers.

Cyril G. Hopkins, Chief in Agronomy

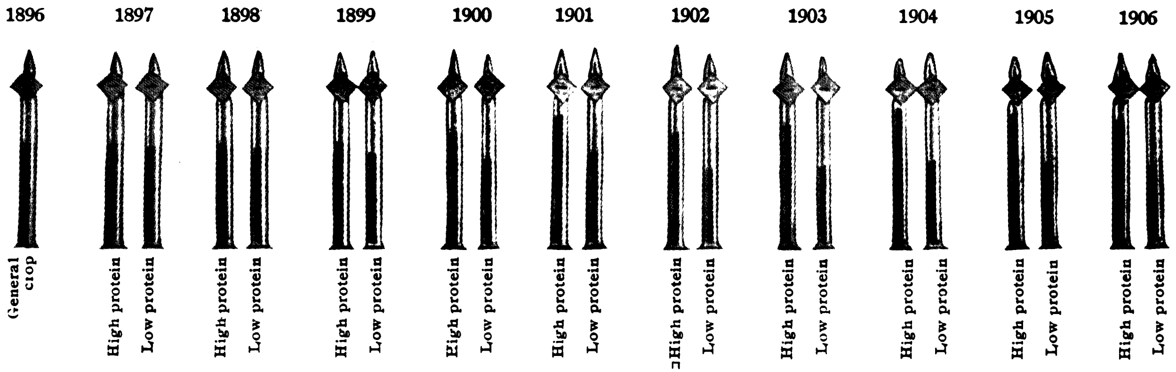

Figure 1. Ten years corn breeding for high and low protein.

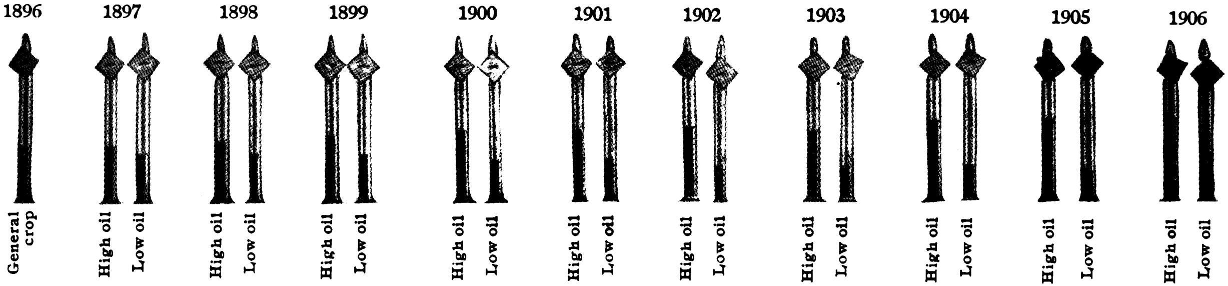

Figure 2. Ten years corn breeding for high and low oil.

Figure 1 illustrates the results of breeding corn for increase and decrease of protein content; Figure 2 for increase and decrease of oil content. The bottles show the amount of protein or oil contained in one-tenth bushel of corn from the crop of the different strains each season for the first ten generations.

Ten Generations of Corn Breeding

L. H. SMITH

SUMMARY

1. The results of 10 years experiments in breeding corn to modify the composition of the grain and thereby adapting it to various special purposes are here reported.

2. Starting with a variety of average composition, it has been possible by selection and breeding, in ten generations: (1) to increase the average protein content from 10.92 to 14.26%; (2) to decrease the average protein content from 10.92 to 8.64%; (3) to increase the average oil content from 4.70 to 7.37%; (4) to decrease the average oil content from 4.70 to 2.66%. In other words, out of a single variety of corn two strains have been developed of which one is now almost twice as rich in protein as the other, and two other strains have been developed, one of which is now nearly three times as rich in oil as the other.

3. Variations among individual ears have been found ranging in protein content from 6.13% in the low-protein strain, to 17.79% in the high-protein strain, and in oil content from 1.60% in the low-oil strain to 8.59% in the high-oil strain.

4. Climatic conditions exert, in certain years, a marked effect upon the composition of the corn crop as regards its protein, oil, and starch content.

5. Altering the composition of the grain has produced no very marked effect upon the composition of other parts of the corn plant.

6. Continued selection appears to have induced a certain correlation between protein and oil content.

7. Selection for the composition of the grain has resulted in characteristic types of kernel.

8. Perceptible modifications in the type of ear have likewise been wrought.

9. Selection for high-protein is evidently accompanied by a reduction in yield. In the other strains the yields for the most part have been maintained in spite of the rigorous selection for the special chemical characteristics.

10. The detailed plot records of each of the four strains and the analytical results of nearly 5000 individual ears which have been analyzed during the 10 years' work are placed on record in the appendix to this bulletin in such arrangement that the maternal pedigree record of every ear is shown.

11. These four breeding plots are still being continued.

[Editor's Note: Appendix, which appeared in original bulletin, has been deleted. Page numbers for each of these numbered items have also been deleted.]

IMPORTANCE OF CORN IMPROVEMENT

Aside from the purely scientific interest attached to this work, the practical importance of improvement of corn to adapt it for special purposes as well as for increased yield is now becoming generally recognized. The significance of improving the chemical composition of corn has already been pointed out in Bulletins 55, 82, 87, and 100 of the Illinois Experiment Station, and it is scarcely necessary to dwell upon this phase of the subject further than to refer briefly to some of the demands for corn improved along these particular lines.

No other crop is made to serve such a variety of purposes as corn, and in consideration of these many different uses is suggested the question of special adaptation.

Purpose of Increasing the Protein. In the nutrition of man and beast protein is the most expensive nutrient. Of all of our American food stuffs corn is the cheapest, because of its economical production. But because corn does not contain sufficient protein for most purposes of feeding, it must be re-inforced by other more expensive food stuffs in order to obtain the proper ratio of this important nutrient. It is from these considerations that farmers, and especially stock feeders, recognize the importance of breeding corn for increase of protein content.

Purpose of Decreasing the Protein. On the other hand, there is a demand from the manufacturers of those products which are derived from the starch of corn such as glucose, gum, dextrine, syrup, and alcohol, for a corn having a large proportion of carbohydrates and not so rich in protein. The practical effect of decreasing the percent of protein is to increase the percent of starch; therefore, for such purposes there should be a place on the market for corn which is bred for decrease of protein content.

Purpose of Increasing the Oil. The oil of corn has in recent years found such a wide commercial use that under the present market conditions, it has become, pound for pound, by far the most valuable constituent of the grain, and whereas formerly in the glucose factories and corn mills the germs containing the oil were almost a waste product, there is now an actual demand on the part of these industries for corn which is richer in oil. It is proposed to meet this demand by breeding corn for increase of oil content.

Purpose of Decreasing the Oil. There is also a practical use for corn with a low oil content. It has been found by investigation that in feeding swine, the oil in the corn tends to produce a soft, flabby quality of flesh which is very undesirable, especially for our export trade where the demand of the market is for a hard, firm product. A remedy for this lies in the reduction of the oil content of the corn which is fed. Thus here we have a very important practical object for breeding corn for decrease of oil content.

These special purposes mentioned for which corn is being improved suggest the possibility of many others demanded by the various industries which utilize the corn crop and which require different qualities in it.

Corn improvement should, of course, embrace quantity as well as quality and in all practical work of selection looking toward improvement, the matter of increased yield per acre should be given first consideration. Recognizing the importance of this principle, the methods used in these experiments have been chosen with the view of maintaining or increasing the yield, and productiveness is made the basis of the first selection, even sometimes at a sacrifice in percentage of the desired chemical constituent.

FUNDAMENTAL PRINCIPLES

Before taking up this work of the improvement of corn by systematic selection and breeding, it was necessary to make a preliminary study of the subject such as is reported by Doctor Hopkins in Bulletin 53 "Chemistry of the Corn Kernel." In this study a large amount of valuable knowledge was gained which bears upon the technical side of the work, such as the chemical principles involved and the laboratory manipulations upon which the success of the entire work so intimately depends. Further, important data were obtained from which were derived the principles of selection upon which all of this work in improvement of the composition of corn is based. All improvement by selection and breeding depends, of course, upon variation, therefore it was necessary to make a preliminary study in order to learn how corn varies with respect to its composition. As the result of such an investigation the following data were obtained:

Analysis of Parts of the Ear. In studying this question 30 duplicate analyses were first made on different parts of ears. Five ears were divided lengthwise into 3 samples each in the following manner: If the ears were 12-rowed, 3 samples of 4 consecutive rows each were made; if 16-rowed, 3 samples of 5 consecutive rows each were made, one row being left, etc., etc.

Duplicate analyses of 15 samples thus prepared from 5 different ears gave the results shown in Table 1. The different ears are distinguished by the letters (a), (b), (c), (d), and (e).

These results indicate uniformity in the composition of different parts of the ear. The following shows the greatest total variation in the 6 single determinations of each constituent in any one ear; and also the total variation between the different ears.

	Ash	Protein	Oil	Carbohydrates
In any single ear	.09	.58	.28	.55
In five ears	.24	2.13	1.09	2.86

Another lot of five ears was selected and each of these was divided crosswise into 3 samples of approximately equal amounts, which for con-

Table 1. Variation in composition in samples from the same ear and from different ears.

Sample & Ear.	Ash.	Protein.	Oil.	Carbohydrates.
1 (a)	1.42	10.79	4.57	83.22
	1.43	10.75	4.58	83.24
2 (a)	1.48	10.97	4.54	83.01
	1.47	10.94	4.51	83.08
3 (a)	1.50	10.66	4.53	83.31
	1.51	10.72	4.55	83.22
4 (b)	1.51	12.00	4.60	81.89
	1.52	11.98	4.59	81.91
5 (b)	1.49	12.01	4.57	81.93
	1.48	12.05	4.57	81.90
6 (b)	1.48	12.19	4.85	81.48
	1.47	12.08	4.80	81.65
7 (c)	1.37	10.09	5.24	83.30
	1.37	10.10	5.17	83.36
8 (c)	1.31	10.14	5.08	83.47
	1.34	10.18	5.18	83.30
9 (c)	1.36	10.15	5.20	83.29
	1.37	10.20	5.17	83.26
10 (d)	1.39	10.46	4.28	83.87
	1.38	10.46	4.29	83.87
11 (d)	1.43	10.25	4.22	84.10
	1.42	10.27	4.20	84.11
12 (d)	1.43	10.09	4.16	84.32
	1.45	10.06	4.15	84.34
13 (e)	1.34	11.19	4.80	82.67
	1.36	11.20	4.78	82.66
14 (e)	1.30	10.66	4.91	83.13
	1.28	10.62	4.89	83.21
15 (e)	1.36	10.81	4.83	83.00
	1.36	10.92	4.79	82.93

venience are designated "tip," "middle" and "butt," the ears being lettered (f), (g), (h), (i) and (j). The results of the duplicate analyses are given in Table 2.

It is observed that in every case the tip is the lowest in protein and that usually the middle is lower than the butt, the average total difference in the ear being 0.73% and the widest 1.13% as shown in the total variations following Table 2.

The variation in ash and oil is small and shows no such peculiarity. The carbohydrates, being determined by difference, appear, of course, as the complement to the sum of the other substances and show in the opposite direction approximately the variation of the most variable determinable constituent.

Table 2. Variation in composition in butt, middle and tip portions of the same ear and of different ears.

Sample & Ear.	Ash.	Protein.	Oil.	Carbohydrates.
16 (f)	1.58	11.78	5.09	81.55
Tip	1.59	11.76	5.10	81.55
17 (f)	1.58	12.22	5.13	81.07
Middle	1.57	12.26	5.03	81.14
18 (f)	1.56	12.36	5.04	81.04
Butt	1.58	12.42	5.03	80.97
19 (g)	1.49	11.99	4.86	81.66
Tip	1.49	11.97	4.84	81.70
20 (g)	1.51	12.49	4.77	81.23
Middle	1.51	12.49	4.76	81.24
21 (g)	1.50	13.02	4.57	80.91
Butt	1.51	13.10	4.59	80.80
22 (h)	1.37	9.72	3.90	85.01
Tip	1.35	9.67	3.93	85.05
23 (h)	1.37	10.07	3.98	84.58
Middle	1.35	10.08	3.97	84.60
24 (h)	1.51	10.49	4.01	83.99
Butt	1.49	10.46	4.00	84.05
25 (i)	1.47	10.58	4.58	83.37
Tip	1.48	10.61	4.60	83.31
26 (i)	1.45	11.05	4.56	82.96
Middle	1.44	11.03	4.60	82.93
27 (i)	1.47	11.03	4.48	83.02
Butt	1.48	10.96	4.46	83.10
28 (j)	1.77	10.87	4.36	83.00
Tip	1.74	10.78	4.37	83.11
29 (j)	1.65	11.35	4.56	82.44
Middle	1.62	11.31	4.58	82.49
30 (j)	1.71	11.32	4.28	82.69
Butt	1.72	11.28	4.29	82.71

The following shows the total variation:

	Ash	Protein	Oil	Carbohydrates
In any single ear	.16	1.13	.30	1.06
In five ears	.42	3.43	1.23	4.25

Partial Analyses of Single Kernels. For the work on ash content several ears of corn were selected, and from each a sample of corn, consisting of a number of rows of kernels and believed to fairly represent the ear, was taken and its percentage of ash in the dry matter determined. Then for special investigation of ash content of single kernels four ears from the lot were chosen, of which two were high and two low, comparatively, in percentage of ash as previously determined. From each ear 10 kernels were selected at approximately equal distance throughout the length of the ear, the kernels being numbered from 1 to 10 and the order running from tip to butt. The data from the ash determination in single kernels and also percentage of ash in the large sample from the same ear are given in Table 3.

These results confirm those of the previous experiments in indicating uniformity in the composition of the ear in all parts, although, of course, slight variations are found.

In the work on the protein content of single kernels, 5 ears, 3 of which were high and 2 relatively low in protein, were selected from a number of ears in a manner analogous to that described in the previous experiment. In Table 4 are shown the results of these protein determinations.

Here in the protein content, as in the case of the ash, we find on the one hand comparative uniformity among different kernels of a single ear and on the other, marked variation among different individual ears.

The results of these analyses of different ears from a single variety together with analyses of different parts of single ears establish beyond ques-

Table 3. Variation in ash content in kernels from the same ear and from different ears.

Kernel No.	Ear No. 1	Ear No. 2	Ear No. 3	Ear No. 4
1	1.50	1.64	1.10	1.14
2	1.57	1.64	1.08	1.23
3	1.61	1.63	1.09	1.13
4	1.56	1.65	1.10	1.17
5	1.67	1.59	1.07	1.13
6	1.69	1.63	1.09	1.22
7	1.71	1.68	1.07	1.25
8	1.64	1.65	1.10	1.19
9	1.64	1.70	1.21	1.11
10	1.74	1.60	1.11	1.10
Composite of ear	1.73	1.65	1.10	1.11

Table 4. Variation in protein content in kernels from the same ear and from different ears.

Kernel No.	Ear No. 1	Ear No. 2	Ear No. 3	Ear No. 4	Ear No. 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	14.59	12.95	8.70	8.76
7	12.50	13.21	12.84	8.46	8.89
8	12.14	13.43	.....	8.69	9.02
9	12.14	13.16	12.04	8.86	8.96
10	12.71	14.05	12.75	8.10	8.89
Composite of ear	13.06	13.87	12.96	7.59	8.40

tion two important fundamental facts upon which all of this subsequent work of selection and breeding is founded.

The statement of these facts is as follows:

(1) *The ear of corn is approximately uniform throughout in the chemical composition of its kernels.*

(2) *There is a wide variation in the chemical composition of different ears of the same variety of corn.*

With these two principles established, we have a working basis for the chemical selection of seed corn. With uniformity in the individual ear, it is possible to determine very approximately the composition of the grain by analyzing a sample consisting of a few rows of kernels, and this is the actual practice in the examination of individual ears. If the ear represented by this sample is found to be desirable for seed, the remainder of the kernels of the ear may then be planted.

The wide variation in composition between different ears of the same variety is a very important factor in the selection of seed; as a starting point is thus furnished in each of the several lines of desired improvement.

It is to be observed that this principle of uniformity within the individual, and variation as between different individuals within the variety, holds not only for the chemical composition of the kernel but it applies as well to other characteristics such as the structure, for example, and whenever any such characteristic is related to productiveness or other utility of the crop, it should be taken into account in the breeding.

GENERAL PLAN OF THE EXPERIMENTS

In the general plan of these experiments, it was proposed to determine the influence upon the chemical composition of corn by selection and breeding in the four directions namely, (1) for increase of protein content, (2) for decrease of protein content, (3) for increase of oil content, (4) for decrease of oil content.

The method employed was as follows:

For the first selection a large number of ears were analyzed both for protein and for oil. In the high protein breeding, for example, the 24 ears highest in protein were selected for seed and planted in a plot isolated from other sorts of corn, each ear in a separate row.

These rows were harvested separately and the seed for the next planting was selected from ears of this crop which were found to be highest in protein, repeating this process each year. The breeding for low protein and for high oil and low oil was conducted on the same plan. Under this system each selection rapidly gave rise to a "pure" strain. As each original ear had its own register number and as all succeeding ears bore corresponding numbers the exact pedigree of each row (on the female side) was at all times fully known.

This general method has been maintained from the beginning, although some minor modifications of details have been made from time to time during the progress of the work as experience indicated as being desirable or as necessity demanded.

The Breeding Plot

The size of the breeding plot has varied in the different plots and in the different years. The number of rows included in any plot is always given in the plot records.

The locations of these breeding plots have always been chosen with reference to their isolation from other corn fields in order to prevent cross fertilization from other kinds of corn. It is quite difficult with so many corn experiments as are carried on at this Experiment Station to obtain conditions that are ideal in this respect, but by taking advantage of tall hedge rows, and other barriers, prevailing winds, and other corn fields of the same strain, there has been but slight, if any, admixture in these breeding plots. A system of alternating the locations of the breeding plots of the opposite strains has been carried out. For example, after the first two years the locations of the high-protein and low-protein plots were reverse; that is the high-protein plot was planted on the same ground that the low-protein plot had occupied the two preceding years and *vice versa.* After two years more these plots were shifted back to their original locations. The high-oil and low-oil plots were managed in the same manner. The design of this alternation of location of the plots was to provide something of a check upon the possible influence of soil upon the composition of the crops. The plots have been changed to other locations in later years but this system of alternating has been maintained. For each of these breeding plots there is now provided a double area which makes possible a crop rotation system including clover and other legumes, for maintaining the productivity of the land.

Cultural Conditions

The cultural methods, including the preparation and cultivation of the soil, planting harvesting, and handling of the crop on these breeding plots have been such as is considered good practice in ordinary corn growing. The seed has always been planted in hills in preference to drills. The present practice is to plant the hills three feet apart each way and to allow two stalks to the hill.

Attention is paid to the matter of preventing the distribution of pollen from weak, barren or otherwise undesirable plants by detasseling all such plants at the proper time.

After discovering the great advantage to be gained by the method of detasseling alternate rows and taking seed only from such detasseled rows, as pointed out in Bulletin 100, this system has been applied to all of our regular breeding plots.

The method of harvesting has been that of cutting and curing in the shock.

Sampling and Selecting

In the earlier years of the experiments a sample from each of all of the rows was reserved by selecting a certain number of the choicest ears as judged from their physical appearance. But as the possibility of improvement became more clearly established, a system was adopted by which seed ears for the next year's planting are taken only from those rows which prove to be most productive as determined by the weight of ear corn produced, all other rows of the plot being rejected as a source of seed, and since the introduction of the system of detasseling alternate rows, only the best of the detasseled or "dam" rows have been selected. This method of selecting the choicest ears to represent the plot-row has been followed throughout the work although details of the system as regards the number of ears taken have been somewhat modified in the different years, as will appear in connection with the data which follow in the appendix.

The ears, thus chosen on account of their physical superiority, are then subjected to chemical analysis and from the results of these analyses is made the final selection of seed for the succeeding season.

In the sampling for these analyses two rows of kernels are taken lengthwise of the ear to represent the composition of the individual ear. At the same time composite samples to represent the selected plot-rows are taken by mixing together one row of kernels from each selected ear of the respective plot-rows. Each seed ear thus selected is given a permanent "Register Number" which designates that ear for all future reference.

Registering

By our system of numbering the "Register Number" shows at the same time the number of the ear and the generation of the breeding. This is done by starting the first year in the 100 series numbering the ears to be planted in succession from 101, and the second generation starting with the 200 series running up from 201 and so on, starting each succeeding year of the breeding with a higher hundred. Thus Ear No. 1018 shows that this ear belongs to the tenth generation and was planted in row 18 of the breeding plot of that year. The "Dam No." is the register number of the parent ear and is useful in tracing the pedigree record from year to year. The "Annual Ear No." is simply a temporary number given to each ear to be used during examination for selection and as soon as the selection of the seed ears has been determined and the arrangement for planting has been decided the ears are given their permanent register numbers.

A description of the physical as well as the chemical characteristics of all the seed ears is kept on record including length of ear, tip circumference of ear, butt circumference of ear, number of rows of kernels, number of kernels in row, weight of ear, weight of cob, tip circumference of cob, and butt circumference of cob. Besides this numerical description a photograph record is also kept of every ear planted.

The performance record of each seed ear is shown by the weight and number of ears produced as well as the average composition of its progeny.

For a more detailed description of the system of registry used in our corn breeding work the reader is referred to Bulletin 100.

Variety

The variety of corn selected for this investigation was one of medium size and of safe maturity for this latitude. It has been grown upon the Experiment Station farm every year since 1887. Previous to that time it had been carefully grown for several years by Mr. F. E. Burr of Champaign county, and it was known locally as Burr's White; and this name was used in our records until 1903, when it was decided to change the name to "Illinois." The fact that these strains of corn are no longer typical Burr's White, and the fact that this corn was carefully grown for several years prior to 1896 by the Illinois Experiment Station and that since that time it has been most carefully bred by this Station for improvement in both yield and quality, so that there have been developed from this variety four different strains of corn each of which has an established pedigree now covering ten generations,—these facts have seemed to justify giving this corn a name which shall be distinctive and which shall also show its Illinois breeding; and now it is known in the records and publications of the Illinois Experiment Station as "Illinois" corn, the four different strains being designated as:

1. "Illinois High-Protein."
2. "Illinois Low-Protein."
3. "Illinois High-Oil."
4. "Illinois Low-Oil."

First Selection of Seed

From the 1896 crop of Burr's White corn grown upon the Experiment Station farm about two bushels (163 ears) of good sound ear corn suitable for seed were taken. From each ear a sample consisting of three rows of kernels lengthwise of the ear was taken for analysis. The results of these analyses are given in the first table of the appendix.[1] The data obtained show remarkable variation in the relative proportions of the dif-

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[1] As noted at beginning of this Chapter, Appendix, which appeared in original, has been deleted. References to the Appendix, however, have been retained.

ferent constituents. The ash varies from 1.10 to 1.74%, the protein from 8.25 to 13.87%, the oil from 3.84 to 6.02% and the carbohydrates from 78.92 to 85.70%. This is a good illustration of the variation in composition existing among individual ears of the same variety and indicates something of the possibilities for selection.

According to these variations there were taken from the 163 ears four groups: (1) a set of 24 ears whose percentage of protein was comapratively high, (2) a set of 12 ears each of which contained a low percentage of protein, (3) a set of 24 ears high in oil content, (4) a set of 12 ears low in oil content.

These ears were taken as indicated in the last two columns of Table 15 (Appendix), for the seed with which to start the four respective breeding plots.

It is believed that the interest in this investigation is such as to demand the publication of a complete record of the results in detail, but this data forms such a mass of material as to make it seem advisable to place it in an appendix to this bulletin and to summarize here only the yearly averages which show very well the general results of the work. The reader who may be interested in further detail of the experiments at any point is therefore referred to the appendix where will be found the complete data recorded in systematic arrangement.

BREEDING TO INFLUENCE THE PROTEIN CONTENT

In order to obtain a general survey of these experiments to influence the protein content of corn the following table is compiled from the general averages obtained each generation from the corresponding tables given in the appendix.

From this arrangement of the data we may compare the results of the different seasons and at the same time observe the relations between the two plots, thereby enabling us to follow the progress of the breeding from year to year.

Starting with the crop of 1896 with an average protein content of 10.92%, as represented by the original 163 ears, the average of the seed ears selected for the high-protein plot of 1897 was 12.54% while at the same time low-protein seed ears were selected which averaged 8.96%. The crop harvested from the high-protein plot in 1897 gave an average of 11.10% of

Table 5. Ten generations of breeding corn for increase and decrease of protein.

Year	High-protein plot, average percent protein.		Low-protein plot, average percent protein.		Difference between crops, percent.
	In seed planted.	In crop harvested.	In seed planted.	In crop harvested.	
1896	. ...	10.92	....	10.92	.00
1897	12.54	11.10	8.96	10.55	.55
1898	12.49	11.05	9.06	10.55	.50
1899	13.06	11.46	8.45	9.86	1.60
1900	13.74	12.32	8.08	9.34	2 98
1901	14.78	14 12	7.58	10.04	4.08
1902	15.39	12.34	8.15	8.22	4.12
1903	14.30	13.04	6.93	8.62	4.42
1904	15.39	15.03	7.00	9.27	5.76
1905	16.77	14.72	7.09	8.57	6.15
1906	16.30	14.26	7.21	8.64	5.62

protein while the average of the corresponding low-protein plot was 10.55%. Then selecting again the highest-protein ears out of this year's crop from the high-protein plot, seed for the following year was obtained which averaged 12.49%. Selecting the lowest protein ears from the low-protein plot, the seed for this plot in 1898 averaged 9.06%.

Repeating this process each year the effect has been in a general way to gradually increase or decrease the protein content in the corn according to the selection.

In glancing over the records there are a few irregularities to be seen. Comparing the results of the season of 1898 with that of the preceding year we seem to have lost a little ground in the high-protein breeding, and in the low-protein plot there was no advance made.

The next year however, following a more favorable seed selection in each case, good gains were made in both directions in 1899, and the same is true of the year 1900.

In 1901 the results are abnormal and here we have a striking illustration of the effect which may be produced by the climatic conditions of the season upon the composition of the crop. This year the protein rises abnormally high in the high-protein crop gaining 1.8% over that of the year before and in the low-protein crop, instead of getting the expected decrease this year the protein content rises to over 10%, thus reverting back to a point higher than it had been for two generations. The season of 1901 was an extremely dry one and from the lack of sufficient moisture much of the corn did not properly "fill out." In the formation of the kernel the proportion of protein is greatest in the younger stages of growth and this proportion gradually diminishes as the carbohydrates are deposited. If the conditions are such that this deposition of carbohydrates is checked, as they were this season, the corn comes to maturity with an abnormally large proportion of protein.

In the case of the high-protein plot the damaging effect of this drouth was so pronounced as to render the crop almost a total failure. The yield of ear corn amounted to only about six bushels per acre and consisted mostly of mere nubbins. On account of the scarcity of ears, it was impossible to follow the regular system of sampling, so the entire product from each plot-row was collected and all of the sound ears and even many nubbins were selected for analysis in order to obtain the results of the year and to get any sort of seed with which to maintain the experiment. The composite samples representing the high-protein crop are therefore not obtained from the best 20 ears from each plot-row according to the regular system but they were taken from all of the corn fit to analyze from each row. Thus there were altogether only 60 individual ears from which only five were chosen for seed as being fit to plant. Fortunately it was possible to supplement these with some seed ears from our "Special High-protein" plot which was being carried on for another experiment but which was planted from the same strain as the regular high-protein plot so that these ears could be substituted without disturbing the pedigree record. The low-protein plot did not suffer so badly from the drouth, so that here the sampling and selection were made as usual.

During the season of 1902 the climatic conditions as regards rainfall were just the opposite to those of the previous year and we observe in the results obtained precisely the opposite effect. With the very wet season this year we have a great diminution of protein content in the corn in the high-protein as well as in the low-protein plot.

This seasonal condition which seems to have such a marked influence upon the composition of the corn is quite significant. The season of 1901 was very dry and it was attended by an abnormally high protein content in all the corn examined that year. The season of 1902 was unusually wet and the general tendency was to produce corn low in protein. These results are in accordance with those of other investigations, particularly in irrigation experiments where it has been observed that the quantity of water supplied has a direct influence upon the composition of corn, wheat, and oats, the protein content of the grain decreasing as the water supply increases. These results support what seems to be a general principle namely, that a lack of moisture tends to increase the proportion of protein and abundance of moisture reduces it, due, of course, to the effect of water supply upon carbohydrate formation.

With a fairly normal season in 1903 the high-protein crop made a notable advance, but the low-protein in spite of the extremely low content of the seed this year did not go down to the point attained in the low-protein season of the previous year, and in fact we have never been able since to bring it back to the extremely low point reached that year.

The season of 1904 appears to have been another one favorable to the production of protein, for the high-protein plot made a gain of 2% this year and reached its maximum figure, 15.03%, a point which has not since been attained. The low-protein plot shows a similar effect, for instead of decreasing this year, it goes up to 9.27 the highest average percentage in the last five generations of the breeding.

In 1905 as might be expected the content in the high-protein crop was not so high as in the preceding high-protein season. In the low-protein plot a good gain was made this year for low-protein.

In 1906 the percentage in the high-protein was still lower than in 1905 while in the low-protein crop, the percentage was a little higher than in the year before although the difference is not great.

The figures in the last column of the table showing the difference between the percentages of protein in the two crops produced each year are perhaps most instructive because they show the real progress attained in the breeding. They enable us to appreciate more fully the scientific value of breeding for high protein and low protein simultaneously and thereby obtaining a control upon the work which serves to eliminate the question as to the effect of seasonal tendencies in either direction.

These figures practically show a continuously increasing separation between the high-protein and the low-protein strains as the breeding advances up to 1906 so that with the exception of two slight regressions, whether the tendency of the season has been toward the production of high-protein corn or low-protein corn, the force of an hereditary influence is demonstrated always to have been in operation.

It is to be recognized of course that there are practical limits both maximum and minimum to which this matter can be carried and we should expect to finally reach a state where we would interfere with the normal physiological functions of the seed.

As to whether this last year's result in which no more gain was made in the difference between the high and low, is to be taken as indicating that we have reached these limits cannot yet be positively decided. It seems scarcely probable that with seed still unimpaired in vitality and developing into normal crops that the ultimate limits should be at hand. It is proposed still to keep up the selection along these lines and the outcome of the next few years will be awaited with interest.

The results of these experiments thus far show that starting with a single variety of corn, it has been possible in ten generations by these methods of selection and breeding to increase the protein content from 10.92% in the original to 14.26%, thus making a gain of 3.34%, and at the same time by breeding in the opposite direction it has been possible to reduce the protein content from 10.92% to 8.64%, making a reduction of 2.28%, thus producing a total difference between the two strains of 5.62%. In other words the composition of this variety of corn has been so modified that two strains have been developed, one of which is now nearly twice as rich in protein as the other.

Mixed-Protein Plot

In order to eliminate the question as to whatever influence the soil might exert on the protein content of the corn an experiment was undertaken in which high-protein and low-protein seed were planted together in one plot, our so-called "Mixed-Protein Plot," where the two strains must develop under identical surrounding conditions.

The description and results of the first year of this experiment are given in Bulletin 55. The first year this mixed-protein plot contained five rows of ten hills each. In each hill were planted two kernels of high-protein corn on one side and two kernels of low-protein on the opposite side in such manner that the resulting plants could be identified.

When the crop was harvested eight to ten ears were selected from each kind of corn from each row and from these ears composite samples were made for analysis. These analyses showed that the average protein content of the corn from the high-protein seed was invariably higher than in that produced from the low-protein seed.

This same experiment was repeated in a somewhat larger plot in 1899 and also in 1900. (See Tables 96, 97 and 98 in the appendix for details).

The differences in protein content between the crops from high-protein and low-protein seed were 1.25% in 1898, 2.58% in 1899, and 2.86% in 1900.

Besides these composite samples there were analyzed from the mixed-protein plot of 1899, 137 pairs of ears in which each pair consisted of an ear produced from a high-protein kernel and one from a low-protein kernel and growing together in the same hill. The results of these analyses are given in Table 99 of the appendix and they show an average difference of 2.58% to be attributed positively to the influence of the seed selection. But with still further interest, it is to be noted that among these 137 different pairs, there are only 10 cases in which the higher percentage of protein is not found in the ear produced from the high-protein kernel. The most notable of these exceptions occurs in case of Row No. 2, Hill No. 11 where the low-protein kernel produced an ear 3.73% higher in protein than the ear resulting from the high-protein kernel. However, these abnormal individual variations are to be expected and they have frequently been observed throughout all of these experiments.

The results of these experiments with the mixed-protein plots during these three different years establish beyond question the fact that the protein content of the corn crop is influenced directly by the seed planted, independently of soil, seasonal, or cultural conditions.

BREEDING TO INFLUENCE THE OIL CONTENT

Summarizing the results of the ten generations of breeding to influence the oil content in the same manner as we have considered the protein breeding, there are brought together from the detailed records in the appendix the general yearly averages of the high-oil and low-oil plots as arranged in Table 6.

The results show that the response to selection for oil has been even more pronounced and more regular than that for protein as indicated by the total relative increase and decrease and by the changes from year to year.

In the percentages representing the crop produced each year in the high-oil plot there has been with but one exception, namely, in 1901, a constant increase in oil content as the breeding proceeds. Likewise in the low-oil plot there has been a steady decrease from year to year with the single exception of the last year.

We have noted the marked effect which the abundance or scarcity of moisture may have upon the protein content of corn, and in these experiments the oil content appears also to be susceptible to some peculiar seasonal conditions. What these conditions are have not been determined, but that they exist is made apparent if we compare the increase and decrease in the percentage of oil in each generation in the two plots. It would appear as

Table 6. Ten generations of breeding corn for increase and decrease of oil.

Year.	High-oil plot, average percent oil.		Low-oil plot, average percent oil.		Difference between crops, percent.
	In seed planted.	In crop harvested.	In seed planted.	In crop harvested.	
1896	....	4.70	....	4.70	.00
1897	5.39	4.73	4.03	4.06	.67
1898	5.20	5.15	3.65	3.99	1.16
1899	6.15	5.64	3.47	3.82	1.82
1900	6.30	6.12	3.33	3.57	2.55
1901	6.77	6.09	2.93	3.43	2.66
1902	6.95	6.41	3.00	3.02	3.39
1903	6.73	6.50	2.62	2.97	3.53
1904	7.16	6.97	2.80	2.89	4.08
1905	7.88	7.29	2.67	2.58	4.71
1906	7.86	7.37	2.20	2.66	4.71

though certain seasons were particularly favorable to the production of oil, while other seasons may be normal or unfavorable in this respect. This effect is particularly apparent in the first 2 years of the breeding; thus, the season of 1897 seems to have been very unfavorable, while the season of 1898 appears to have been very favorable, to the production of oil.

From the last column in the table which shows, by the differences in percentage between the high-oil and the low-oil crops each year, the real progress accomplished by the breeding, we see that there has been a continuously increasing difference between the percentages of oil in the corn from the two plots up to the tenth year where this difference remains stationary. The high-oil corn has increased from 4.70% to 7.37% of oil, and the low-oil corn has decreased from 4.70 to 2.66%, the difference between the two strains having grown from nothing in 1896 to 4.71% in 1905. Curiously enough the oil breeding resembles the protein in the fact that there is constant progress indicated until the tenth year when in each case this progression ceases. In the protein experiments it will be recalled that the figures in in this "difference column" show actually a slight regression in the tenth year while here in the oil breeding the differences between the averages of the high-oil and low-oil crop stands exactly stationary in the last two years.

As has already been remarked in the discussion of the protein breeding results, it would be rash to decide at this time from these figures that the limits to which the breeding can be carried are now determined.

Summarizing the results of the 10 years' experiments to influence the oil content into one general statement we may say that starting with a single variety of corn and breeding in the two opposite directions, there has been a constantly widening separation between the two strains as the breeding advances until finally after ten generations there have been produced two kinds of corn, one of which is almost three times as rich in oil as the other.

Mixed-Oil Plot

In order to eliminate any question of the influence of the soil upon the oil content in these experiments, a third plot was planted called the "mixed-oil plot," after the plan of the "mixed-protein plot" already described under that heading. In 1898 there were planted in this plot 50 hills arranged in five rows of ten hills each. In each hill two kernels of high-oil corn were planted on one side and two of low-oil on the opposite side and when the crop was harvested composite samples were made to represent the corn of each side of the row.

This same experiment was repeated in 1899 and also in 1900 the details being given in Tables 100, 101 and 102 of the appendix.

From the results it is to be noticed that never in any of the rows has the percentage of oil in the crop of the low-oil side approached that of the high-oil side. In 1898 the average difference in oil content in the corn resulting from the two kinds of seed was 1.11%, in 1899 it was 1.35% and in 1900 it was 1.97%.

From the mixed-oil plot of 1899 there were taken besides these composite samples 85 pairs of individual ears in which each pair consists of one ear produced from a high-oil seed kernel and one from a low-oil kernel, both ears from plants growing in the same hill. Each of these individual ears was sampled and analyzed and these results are given in Table 103 of the appendix.

The average of all of the individual ears from high-oil seed is 5.22% and from low-oil seed it is 3.82%. But the point of most interest, perhaps, in connection with this table is the regularity with which the oil content of the crop responds to that of the seed planted, for among the 85 pairs there are only four cases in which the oil in the ear, resulting from low-oil seed happens to surpass in percentage that from the high-oil seed.

The results of these 3 years' experiments with the mixed-oil plot are all in accordance and they establish beyond dispute the possibility of influencing the oil content of corn by the selection of the seed, showing conclusively that heredity has been responsible for the results obtained quite independent of soil, climatic or cultural conditions.

SECONDARY EFFECT PRODUCED BY SELECTION TO CHANGE THE COMPOSITION OF THE GRAIN

As is always the case in investigations of this sort, the work had not proceeded far before a multitude of interesting side questions arose, inviting investigation in all directions from the main issue. What secondary effects are produced by this intense selection for these special chemical characteristics? What, for example, is the effect of changing the proportion of protein in the grain upon the other constituents? How is the composition of other parts of the plant affected? What influence has it upon the physical type of of the kernel and of the ear? And, what is of especially practical importance, how is the yield affected?

Having established the possibility of influencing the composition of the kernel in this way by several years of breeding and after having actually produced the different kinds of corn to work with, it became possible to take up the study of some of these important secondary effects. The results of the investigation of some of these questions are given in the following pages.

Effect on the Composition of Other Parts of the Plant

After the breeding plots had been under way for 5 years and marked changes had been produced, a study was begun to ascertain how the composition of other parts of the plant was being affected by altering that of the grain.

Beginning in 1903, there have been collected every year at harvest time representative plants from each of the four "Illinois" breeding plots. These plants were divided in the following manner into three parts, namely, upper-stalk, lower-stalk, and leaves. The leaves were first stripped off from the stalks and these, including the husks, constituted the sample designated here as "leaves." Then at the joint where the ear was borne, the stalk was divided and the part below this point comprised the sample called "lower-stalk," and all above including the tassel, made up the sample designated as "upper-stalk."

It may be observed that the condition of these samples is just as it would be in the ordinary handling of corn stover on the farm. It was cured in the field in the ordinary manner, the stalks having lost some parts of the leaves and tassels. Then this rather arbitrary division into parts follows somewhat in the natural way in which the stover is eaten by animals as fed entire without cutting or shredding. The leaves and husks are entirely consumed and usually a portion at least of the upper stalk is eaten. If any is refused, it is the coarser part of the stalk corresponding somewhat to our sample of "lower-stalk." With this practical bearing in mind, there is lent something of an added interest to the analyses.

The results of the analyses of these samples are brought together in the tables that follow. For convenient comparison each constituent is considered by itself in a table showing the percentages found in the several parts of the plant in the different strains each season.

In the first 2 years of the work these samples were taken from every individual breeding row in the four plots so that the results shown here really represent averages of several hundred analyses, but these data form such a mass of material that lack of space forbids presenting them here in detail.

Effect on the Ash Content

We will consider first the effect produced by the breeding upon the ash content as shown in Table 7.

Comparing the percentages of ash in the high-protein and low-protein strains in the upper-stalk, there is no regularity apparent. In two of the sea-

Table 7. Ash content in different parts of plant.

Year.	Strain.	Upper-stalk.	Lower-stalk	Leaves.	Grain.
1902	High-Protein........	5.25	4.08	8.21	1.57
	Low-Protein	5.82	5.09	8.64	1.45
	High-Oil............	5.65	4.89	7.59	1.54
	Low-Oil.............	4.91	3.72	7.11	1.42
1903	High-Protein........	5.23	4.52	9.66	1.52
	Low-Protein.........	4.86	4.28	7.98	1.34
	High-Oil............	5.20	3.98	8.23	1.47
	Low-Oil.............	4.75	4.27	7.27	1.47
1904	High-Protein........	4.38	3.95	6.56	1.60
	Low-Protein.........	5.14	4.57	7.51	1.41
	High-Oil............	5.05	5.80	7.66	1.56
	Low-Oil.............	5.59	5.53	8.12	1.43
1905	High-Protein........	4 30	4.02	6.52	1.54
	Low-Protein.........	5.00	4.61	7.47	1.50
	High-Oil............	5.26	5.92	8.06	1.58
	Low-Oil.............	5.69	5.47	8.34	1.28
1906	High-Protein........	4.77	4.93	9.08	1.48
	Low-Protein.........	4.28	4.15	8.85	1.41
	High-Oil............	5.05	5.61	7.72	1.64
	Low-Oil.............	5.67	4.84	7.01	1.46

sons the percentage was greater in the high-protein plot and in the three other seasons it was smaller. The lower-stalk varies in the different seasons in accordance with the upper-stalk, and the same is true of the leaves. In the grain the differences are very slight but they show every season a little more ash in the high-protein corn.

Comparing the samples of the various parts from the high-oil and low-oil strains, we find no regularly concordant variations except in the case of the grain where usually the percentage of ash has been a trifle higher in the high-oil than in the low-oil corn. In regard to the distribution of the ash over the plant as a whole we find, as we should expect in accordance with what is generally observed in plant studies of this nature, the lowest proportion of ash in the seed and the highest in the leaves where it amounts sometimes to almost one-tenth of the dry substance.

Effect on the Protein Content

It is especially interesting to note how the change in the proportion of protein in the grain has affected this constituent in other parts of the plant. For example, does the increase of protein in the kernel mean an increase of this substance in the other organs of the plant, or is this higher content in the kernel the result of an accumulation produced at the expense of other parts?

Table 8. Protein content in different parts of plant. (Protein derived by multiplying the nitrogen content by the factor 6.25.)

Year.	Strain.	Upper-stalk.	Lower-stalk	Leaves.	Grain.
1902	High-Protein........	3.31	3.28	5.00	12.34
	Low Protein.........	2.90	3.21	4.99	8.22
	High-Oil.............	3.70	4.72	5.13	10.83
	Low-Oil.............	2.78	2.83	4.86	9.31
1903	High-Protein........	4.00	3.20	4.92	13.04
	Low-Protein.........	3.80	4.26	5.28	8.62
	High-Oil............	3.20	3.28	5.04	11.04
	Low-Oil.............	3.50	3.58	5.04	10.22
1904	High-Protein........	6.52	5.68	5.34	15.03
	Low-Protein.........	3.06	3.46	4.77	9.27
	High-Oil.............	4.53	4.08	4.86	12.29
	Low-Oil.............	4.00	4.94	5.10	10.88
1905	High-Protein........	6.13	6.03	6.46	14.72
	Low-Protein.........	3.59	4.59	5.81	8.57
	High-Oil.............	4.38	6.32	6.42	12.12
	Low-Oil.............	4.41	4.09	5.74	9.86
1906	High-Protein........	5.99	4.94	5.27	14.26
	Low-Protein...	5.61	6.48	7.13	8.64
	High-Oil.............	5.38	6.56	5.57	11.81
	Low-Oil.............	4.37	4.37	5.03	10.54

Table 8 shows the results of the protein determinations in the various parts of the plant.

Upon comparing the protein content of the upper-stalk samples we find that the percentage has always been greater in the high-protein plot varying in the different seasons, from only a slight difference to over double the amount.

The lower-stalk follows the upper-stalk in this respect in three of the seasons but in the other two years the protein runs higher in the low-protein strain. The leaves agree quite closely in every case with the lower-stalk.

The wide differences in the protein content of the grain are, of course, the direct result of the selection which have already been considered so that we need not discuss them further in this connection.

Turning now to the oil breeding, there seems to be a lack of any significant regularity in the parts of the stover. In the upper-stalk the percentage of protein runs higher three out of the five seasons in the high-oil strain. In the lower-stalk it is three times out of five higher in the high-oil strain but corresponding only three times with the upper-stalk. The leaves correspond with the lower-stalk in this comparison.

But a very marked correlation appears in the grain where there has been every year a notable increase in protein in the high-oil strain over that of the low-oil. This is indeed significant and is of such interest that it will be discussed more fully later on.

Regarding the general distribution of the protein in the plant, the data show; that in the other parts, the proportion of protein is never as high as it is in the grain; that among the other parts, the leaves have averaged somewhat higher than the stalks although this condition has not been constant in every year; that, as between the upper and lower portions of the stalk no regular difference can be established.

Effect on the Crude Fat Content (Ether Extract)

It will be noticed that the term "crude fat" is used here for designating this determination rather than "oil" as has been used in the rest of this work in which only the kernels were concerned. It should be considered that while in the kernels the substance extracted by ether is practically all oil, in the stalk and leaves it consists to a considerable extent of other constituents besides true fats, or oil, such as coloring matters, waxes, organic acids, etc. On this account these results of the ether extraction are not to be taken as necessarily explicitly expressing the relations of the amount of oil or fat in the various organs of the plant to that of the kernels. However in the chemical analyses of food stuffs, the ether extraction is the best practical method that we have at present of classifying these substances, and in view of the fact that it is usually made and reported in fodder analyses this determination was made in this study with the idea that the information would be of interest and might prove suggestive. The results are given in Table 9.

Table 9. Ether extract in different parts of plant.

Year.	Strain.	Upper-stalk.	Lower-stalk	Leaves.	Grain.
1902	High-Protein........	0.98	1.31	1.02	4.85
	Low-Protein.........	0.82	0.95	1.02	4.15
	High-Oil.............	1.08	1.27	0.99	6.41
	Low-Oil.............	0.87	1.21	1.02	3.02
1903	High-Protein........	0.92	1.42	1.04	4.83
	Low-Protein	0.77	0.88	0.90	4.08
	High-Oil.............	0.69	0.86	0.98	6.50
	Low-Oil......	1.18	0.98	0.98	2.97
1904	High-Protein........	0.77	1.23	1.16	5 07
	Low-Protein	0.78	0.81	1.00	4.17
	High-Oil.............	0.67	0.87	1.10	6.97
	Low-Oil.............	0.69	0.90	0.98	2.89
1905	High-Protein........	0.95	1.20	1.21	5.04
	Low-Protein	0.88	0.95	1.28	3.85
	High-Oil.............	0.82	1.00	1.24	7.29
	Low-Oil.............	0.72	0.82	1.09	2.58
1906	High-Protein........	1.02	1.69	1.25	5.28
	Low-Protein	1.62	1.89	1.46	3.86
	High-Oil.............	1.07	2.05	1.32	7.37
	Low-Oil.............	1.29	1.46	1 23	2.66

The proportion of ether-extract in the stalks and leaves is not very large ranging mostly around 1% and there are no apparent relations among these results that would indicate any significant influence of the selections either in the protein or in the oil breeding.

In the case of the grain, however, there is an interesting correlation manifested. We have already seen how the protein content of the grain is influenced by the oil selection, and, just as the protein rises and falls with the oil content, so here the oil follows the protein selections, and in every season there is a decided increase of oil in the high-protein strain over that of low-protein.

The figures show in regard to the relative proportions of ether-extract in the different parts of the plant, that the crude fat in other parts scarcely ever approaches in percentage the oil in the kernel and also that it is generally greater in the lower-stalk and leaves than in the upper-stalk.

Effect on the Phosphorus Content

On account of their especial bearing upon questions pertaining to soil fertility a knowledge of the phosphorus and potassium contents in these different strains of corn is of interest. Accordingly determinations of these two elements in the samples of the different parts of the plants have been made since 1903. The percentages are given in Tables 10 and 11 being expressed in terms of the elementary substances.

Comparing the high-protein and low-protein strains it is interesting to note that with only the exception of the upper-stalk and leaves in 1906, the phosphorus content is always somewhat higher in the samples representing the high-protein plot both in stover and grain.

Table 10. Phosphorus content in different parts of plant.

Year.	Strain.	Upper-stalk.	Lower-stalk	Leaves.	Grain.
1903	High-Protein........	0.19	0.19	0.15	0.36
	Low-Protein.........	0.12	0.10	0.14	0.30
	High-Oil............	0.10	0.08	0.10	0.34
	Low-Oil.............	0.10	0.09	0.13	0.31
1904	High-Protein........	0.26	0.24	0.18	0.38
	Low-Protein.........	0.14	0.10	0.12	0.33
	High-Oil............	0.21	0.12	0.16	0.38
	Low-Oil.............	0.17	0.17	0.18	0.35
1905	High-Protein........	0.26	0.22	0.18	0.32
	Low-Protein.........	0.12	0.09	0.12	0.30
	High-Oil............	0.18	0.11	0.12	0.34
	Low-Oil.............	0.17	0.14	0.17	0.25
1906	High-Protein........	0.28	0.32	0.21	0.34
	Low-Protein.........	0.29	0.21	0.24	0.30
	High-Oil............	0.25	0.22	0.19	0.35
	Low-Oil.............	0.19	0.14	0.15	0.31

Table 11. Potassium content in different parts of plant.

Year.	Strain.	Upper-stalk.	Lower-stalk	Leaves.	Grain.
1903	High-Protein........	1.47	1.64	0.90	0.35
	Low-Protein.........	1.52	1.64	0.97	0.32
	High-Oil.............	1.34	1.10	0.76	0.36
	Low-Oil.............	1.33	1.54	1.25	0.36
1904	High-Protein........	1.07	1.10	1.02	0.37
	Low-Protein.........	1.67	1.67	1 31	0.35
	High-Oil.............	1.63	1.70	1.48	0.39
	Low-Oil.............	1.55	1.74	1.56	0.39
1905	High-Protein........	1.03	1.07	1.05	0.34
	Low-Protein.........	1.61	1.62	1.35	0.37
	High-Oil.............	1.65	2.36	1.39	0.36
	Low-Oil.............	1.81	2.08	1.60	0.37
1906	High-Protein........	1.17	1.54	0.88	0.36
	Low-Protein.........	0.89	1.17	0.82	0.40
	High Oil.............	1.69	2.14	1.22	0.39
	Low-Oil.............	1.59	1.79	0.92	0.40

In the breeding for high and low-oil, however, such a correlation does not appear in the stover but in the grain we find regularly a higher phosphorus content in the high-oil corn. Taking the plant as a whole the grain is decidedly the richest part in phosphorus thus conforming to what has been generally observed.

Effect on the Potassium Content

Comparing the high-protein and low-protein plots the different parts of the stover show agreement in three out of the four years in being somewhat richer in potassium in the low-protein strain. The other season all parts were richer in this element, in the high-protein strain.

In the grain samples the comparisons show conflicting results but the differences here are so small as to be scarcely significant.

As between the high-oil and low-oil breeding no regularity among the stover samples can be made out. In the grain the percentages are just a trifle greater in the low-oil corn but the differences are too slight to be considered seriously.

These results likewise accord with the usual observance that the stover carries a much larger proportion of potassium than the grain. The stalks and leaves do not vary greatly in this respect.

Conclusions

The preceding data afford material for numerous other comparisons and a critical study would doubtless reveal many other suggestive facts, but it is our present purpose only to derive as direct an answer as possible to our main question regarding the effect produced upon the composition of the

Table 12. Oil content in high-protein and low-protein strains.

Average percent oil.			
Year.	High-protein crop.	Low-protein crop.	Difference.
1897	4.52	4.35	0.17
1898	....	....	....
1899	....	....	....
1900	4.75	4.31	0.44
1901	4.82	4.30	0.52
1902	4.85	4.15	0.70
1903	4.83	4.08	0.75
1904	5.07	4.17	0.90
1905	5.04	3.85	1.19
1906	5.28	3.86	1.42

plant as a whole by altering the relative proportions of the constituents of the kernel.

Summarizing the results of this study and putting them into the form of a general statement we may say, that aside from the correlation developed between protein and oil in the grain, there has not been produced any very marked effect. The ash in the grain appears to be influenced very slightly by the protein as well as the oil selection, following these selections in direct correlation. The same is true of the phosphorus content.

Further there is seemingly a tendency toward an increased phosphorus content in the stover resulting from high-protein selection, but this observation needs further confirmation.

Correlation Between Protein and Oil in the Kernel

At the beginning of the breeding the correlation between the protein and oil content in the kernel was only very slight. The result of the mathematical calculation of this correlation in the original 163 ears from which the first selections were made, as given in Bulletin 87, shows only 3.81% of a perfect correlation.

But, although this correlation is insignificant at first, it seems to have advanced with the breeding so that, as we have just observed, after five years it became very prominent both in the protein and in the oil selections.

It is interesting to trace the development of this correlation in the progress of the breeding as may be done in Table 12 in which are given the percentages of oil in the high-protein and low-protein strains each generation excepting the second and third years when these determinations were not made.

The last column which shows the difference in oil content each year brings out the principle in a most interesting way. This difference between the two plots begins with a very small figure which gradually increases as the breeding goes on corresponding to the differences in the protein itself, until in the tenth generation this difference becomes so significant as to amount to about one-third of the total quantity of oil.

Table 13. Protein content in high-oil and low-oil strains.

	Average percent protein.		
Year.	High-oil crop.	Low-oil crop.	Difference.
1897	10.76	11.03	- 0.27
1898	.....	.....	....
1899	.....	.....	....
1900	10 83	11.00	-0.17
1901	12.32	10.03	2.29
1902	10.83	9.31	1.52
1903	11.04	10.22	0.82
1904	12.29	10.88	1.41
1905	12.12	9.86	2.26
1906	11.81	10.54	1.27

In like manner the behavior of the protein in the high and low-oil strains is shown in Table 13.

Although the differences in protein content in the high-oil and low-oil plots have not been as regular or constant as in the case of the oil content in the high-protein and low-protein plots, yet the same principle is evident, for, whereas in the earlier years the protein actually averages a little higher in the low-oil strain as indicated in the table by the minus signs, in later generations after the breeding had advanced and greater differences in the oil content had been induced, the correlation appears and remains, although fluctuating in intensity from year to year.

Effect on the Type of Kernel

That the selection for certain chemical constituents has a very noticeable effect upon the physical characteristics of the kernel was observed very early in the work and in Bulletin 55 descriptions with photographs were published showing the possibility of distinguishing between high-protein and low-protein corn as well as between high-oil and low-oil corn by the mechanical structure of the kernel. The matter has been mentioned again in Bulletins 82 and 100, and Bulletin 87, "The Structure of the Corn Kernel and the Composition of its Different Parts," deals especially with this phase of the subject, treating it in considerable detail. Therefore it is not proposed to discuss this matter at length here, but only to call attention briefly to the facts observed in this connection.

Selection for high-protein has developed a type of kernel having a relatively larger proportion of that part characterized by its horny structure, the soft starchy part which immediately surrounds the germ and runs up into the crown of the kernel being less prominent. In the type of kernel resulting from low-protein selection this condition is reversed and here the soft starchy part predominates. Viewed externally the high-protein kernel has a somewhat glassy appearance while the low-protein presents a milky effect.

Following the fact that about four-fifths of all the oil in the kernel resides within the germ, the selection for high-oil has resulted in a kernel having a relatively large proportion of germ, while the low-oil selection has produced a kernel whose germ occupies a relatively small proportion of the space.

It should be borne in mind that a reduction of the proportion of germ does not necessarily depend altogether upon a decrease in the absolute size, for the same effect would be produced by increasing the size of the endosperm, and in our low-oil strain this is what has really taken place to some extent, so that the selection has resulted in a large broad type of kernel admitting fewer rows on the cob.

The question is often asked as to whether there is any difference apparent in germination on account of this effect upon the size of the germ. In laboratory tests under carefully controlled and comparable conditions a difference in the rate and vigor with which the germination starts off has been observed, the first signs of growth appearing about 24 hours earlier in the high-oil corn. This difference, however, becomes less apparent as the development of the young plantlet proceeds and in the field there is as yet no detrimental effect noticeable due to impaired vitality in the seed brought about by the selection for low-oil.

Effect on the Type of Ear

That the selection in these different directions has likewise had its effect upon the physical characteristics of the ear is clearly shown in Bulletin 119, "Type and Variability in Corn," in which Dean Davenport and Doctor Rietz have made this matter the subject of a special study.

In this investigation ears from each of the four "Illinois" strains from the crops of the ninth and tenth generations were subjected to measurements of their length, circumference, weight, and number of rows of kernels.

The variability of each one of these characteristics was studied by the statistical method in which are determined mathematical expressions showing the "mean," or average value of the character in question, as well as its tendency to vary from this average, expressed by the "standard deviation" and the "coefficient of variability." Taking from these tabulated results those figures which are of especial concern in this connection, the following interesting facts are brought out.

Selection for high-protein has produced an ear averaging somewhat smaller than the low-protein ear as shown by a comparison of the length, circumference and weight; the number of rows of kernels also averages slightly less on the typical high-protein ear.

Similarly the high-oil selection has resulted in a smaller type of ear than has the low-oil, the length, circumference and weight being less in each case in the high-oil strain. However, in spite of the fact that the typical low-oil ear is the largest of any of the strains the number of rows of kernels is the least, this being due to the broadening of the kernel as previously explained.

Effect on the Yield

One of the first questions to be taken into consideration from the practical standpoint is, of course, the effect that selection for these various characteristics has upon the productiveness.

In this connection it should be borne in mind that during the earlier years of these experiments in the selection of seed no special precaution was taken against in-breeding. If the pedigree lines be traced back in the high-protein plot it will be found that they all converge in a single ear grown in 1901. The low-protein strain as it now exists is the progeny of two of the original ears and the same is true of the low-oil. The high-oil strain traces back to three original ears. Thus the pedigree records show that there must have been a considerable amount of rather close in-breeding which has probably exerted a more or less detrimental effect upon the yield. It was not until the ninth generation that we started our present system of taking seed from detasseled rows only and arranging the planting of the seed ears with reference to their relationship, in order to prevent as far as possible such close in-breeding.

Neither was there in the earlier years of the breeding any selection based upon productiveness other than the choice of the largest, finest seed ears. In the sixth generation a system of rejecting a few of the lowest yielding rows was begun but it was not until the ninth generation that our present system was adopted of selecting one-half of the detasseled rows according to their performance as regards productiveness.

Therefore in speaking of the yielding propensities of these several strains of corn, these handicaps which they have undergone in the breeding should be taken into consideration.

In order to test this matter of yield, seed has been taken every year since the sixth generation from each of the four breeding plots and planted in our variety test plots where they are given conditions of soil and culture as uniform as possible for securing comparable results.

In this variety test there are planted at certain intervals so-called "Standard plots" from one of the best standard varieties of this region the purpose of these being to serve as a check for comparison in different parts of the field. In Table 14 the yield each year in terms of bushels of shelled corn per acre is given for each of the four "Illinois" strains along with that of the standard variety.

In looking over these results there are some irregularities to be seen and it is still too early to draw final conclusions in all respects. The maximum yield varies among the four strains in the different years. In two of the seasons the low-oil gave the highest yield and in two others the low-protein yielded most.

But the lowest yield has in every season been produced by the high-protein corn and this fact accords with our previous observation regarding

Table 14. Yields of "Illinois" strains in variety test plots.

Year.	High-protein strain.	Low-protein strain.	High-oil strain.	Low-oil strain.	Standard variety.
1903	27.3	37.7	32.7	41.3	40.9 (Boone Co. White)
1904	32.1	55.5	41.9	40.5	53.7 (" " ")
1905	56.6	60.7	58.4	58.1	68.4 (Silvermine)
1906	65.1	73.2	66.3	83.2	75.7 (") 87.9 (Leaming)

the type of ear where we found the typical high-protein ear to be the smallest of all the four strains. So it seems a high-protein content and the highest productivity do not go together.

The formation of protein depends, of course, upon the supply of nitrogen in the soil. In fact the relation is so intimate that it has been observed in experiments that the protein content can be increased in corn by the application of nitrogenous fertilizers. This suggests the possibility of a limitation of growth on ordinary soil due to an extra high nitrogen requirement on the part of the high-protein strain.

If, however, we consider the production of *protein per acre* we will find a very decided gain in the production of protein in the high-protein breeding. For example in 1906 the high-protein strain produced 65.1 bushels per acre and the protein content of the crop that year as we have seen was 14.26%. This would yield (reckoning 56 pounds shelled corn per bushel) 520 pounds of protein per acre: At the same time the low-protein strain produced 73.2 bushels carrying 8.54% of protein which would yield 354 pounds. This makes a difference of 166 pounds of protein per acre in favor of the high-protein breeding. This, however, from the practical standpoint, would be an unfair comparison because ordinarily what the farmer has to deal with is corn of ordinary protein content rather than low-protein corn. We have no "Illinois" strain now unaffected by chemical selection with which to make the comparison. But suppose we compare our "Illinois High-Protein" with the standard white variety for this year, that is the "Silvermine," which has had no chemical selection, and assume that it contains the same percentage with which we started the "Illinois" breeding, that is 10.92, which as a matter of fact is not far from the average of ordinary dent corn. Making the computation we find that the 75.7 bushel yield containing 10.92% would give us 463 pounds of protein per acre. Based upon this estimate there was a gain this year of 57 pounds of protein per acre by the high-protein breeding and this is of no mean consequence when we consider that this 57 pounds represents about one-eighth of the total quantity of protein produced.

On the whole these results of the yields are quite gratifying when we consider that these "Illinois" strains have maintained their productiveness as well as they have in spite of the intense selection they have undergone for other special characteristics. With the exception of one season, some one of the Illinois strains has even surpassed in yield the supposedly good variety used as a standard. All of this goes to show that intense selection for a special character is not necessarily accompanied by a reduction in yield, this not implying, of course, that selecting for yield alone would not make greater progress when unhampered by consideration for other characteristics.

4

The Mean and Variability as Affected by Continuous Selection for Composition in Corn

F. L. WINTER

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INTRODUCTION

The effectiveness of selection in cross-fertilized crops such as maize may be explained upon the following principles:

1) There are heritable variations existing among the different individuals of the population at the beginning.

2) There is a gradual elimination of the undesirable type because the selected types consist of more desirable and fewer undesirable individuals in each generation.

3) Desirable mutations which may occur are retained and caused to combine with the desirable factors present.

4) Recombination of the desirable factors produces more desirable types.

Selection for a given type not only tends to bring the population to that type but is expected to decrease the variability. This decrease in variability of the population is brought about by a reduction in the percentage of heterozygous individuals. After the population becomes homozygous for the selected character, no further reduction in variability through selection can be expected. The variability that still remains is attributed to environment, upon which selection has no influence.

It is the purpose of this paper to present the effect of 28 years of continuous selection for composition in maize upon the mean and the variability of the selected character.

MATERIAL

In 1896 a series of breeding experiments was begun at the Illinois Agricultural Experiment Station to determine whether the chemical composition of corn could be influenced by selection (8). One hundred and sixty-three ears of a variety known as Burr's White were used as the foundation stock, from which selections were made in four different directions, namely, for high oil, low oil, high protein, and low protein.

These four strains were carried on in the same way. In the high protein, for example, the 24 ears highest in protein were selected for seed and planted in an isolated plot, each ear in a separate row. These rows were harvested separately and the seed for the next crop selected from the ears which were found to be highest in protein. Nine years later the system was modified somewhat in an attempt to prevent loss of vigor by inbreeding. Alternate rows were detasseled and seed was selected only from the highest yielding detasseled rows. In 1921 this system was again modified to reduce the amount of inbreeding. Two seed ears were taken from each of the detasseled rows regardless of yield. The high-oil, low-oil, and low-protein tests were similarly conducted, selection being made each year of ears highest in oil, lowest in oil, and lowest in protein, respectively.

The analytical methods employed have been described in detail in Illinois Agricultural Experiment Station Bulletins 43 and 53 (6, 7).

EFFECT OF SELECTION FOR PROTEIN CONTENT

The effect of selection for protein content in the corn grain is shown in Fig. 1. The average protein content which was 10.92 in 1896 had in 1924 been increased to 16.60% in the high-protein strain and had been decreased to 8.38% in low-protein strain. This is a difference of 8.22%. When measured by the best fitting straight lines, the difference is 9.49%. When compared with the original variety from which the strains were selected, the proportion increase is 50.01% and the proportional decrease is 23.26%.

EFFECT OF SELECTION FOR OIL CONTENT

From an average of 4.70% in 1896, the oil content had been increased to 9.86 in the high-oil strain and decreased to 1.51% in the low-oil strain in 1924. This is a difference of 8.35%. When measured by the best fitting straight lines, the difference is 8.85%. Relatively, selection has been much more effective in bringing about a change in oil content than in protein content. When compared with the original variety, the proportional increase in oil is 109.79% and the proportional decrease is 67.87%. (Fig. 2.)

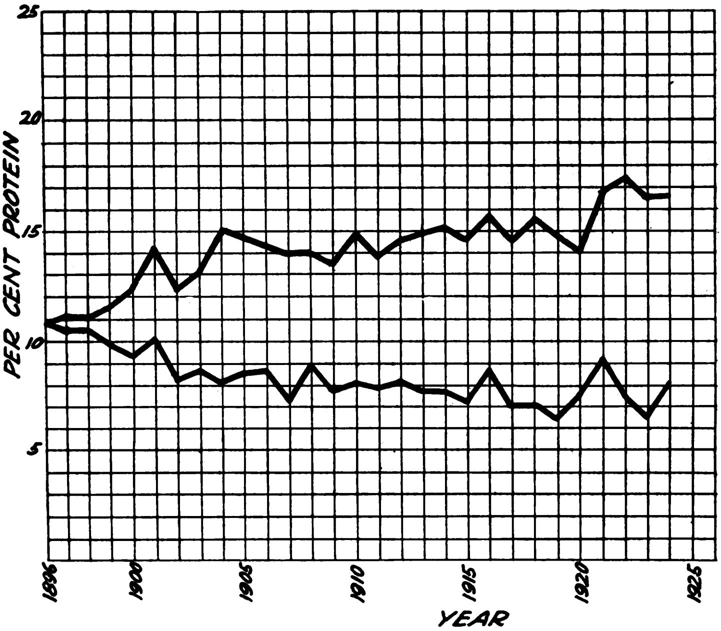

Figure 1. Progress of high-protein and low-protein corn breeding.

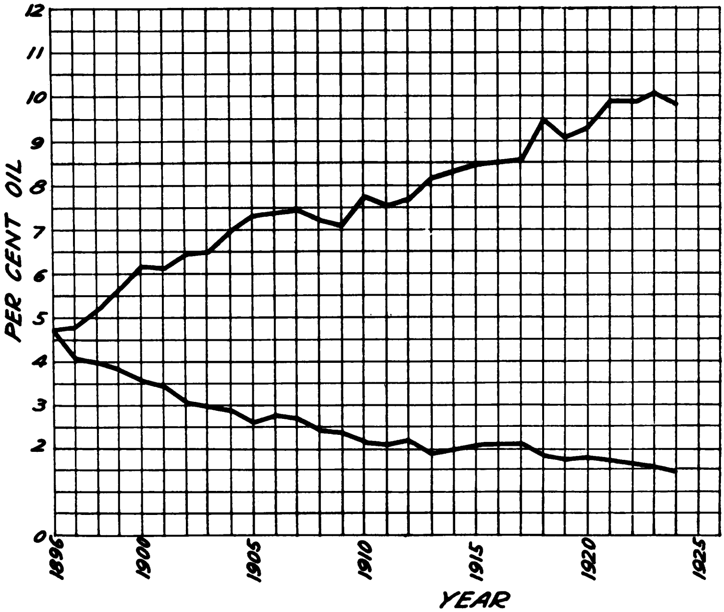

Figure 2. Progress of high-oil and low-oil corn breeding.

It is very probable that a more rapid shift in type with respect to both the oil and protein would have been obtained if only the ears that were highest and lowest in protein or in oil had been selected for seed, regardless of the yielding ability of the detasseled rows. On the other hand, if selection had been carried on without taking yield into account, inbreeding might have resulted in such a loss of vigor that it would have been impossible to continue the strains. When it is remembered that the original purpose of the experiment was to produce good-yielding strains having the desired composition, any great reduction in yield would have defeated the purpose.

PEDIGREES

Graphic presentations of the pedigrees of the strains of corn used in this work are on pages 99 and 100.

A study of the pedigrees of the four strains shows that there has been a rapid elimination of lines until the four strains are now represented by only a single ear each of the original seed stock. The high-protein strain traces back to ear 121 which on analysis showed 12.28% protein and 3.99% oil. This is slightly below the average composition of 12.54% for the stock seed ears but considerably above the average of the original 163 ears, 10.92%. The low-protein strain goes back to ear 106, which on analysis yielded 8.25% protein and 4.81% oil. The average for the stock seed ears of the low-protein strain was 8.96%. The high-oil strain goes back to ear 111, which on analysis showed 5.65% oil and 10.82% protein. The average for the stock seed ears for the high oil was 5.33% as compared to 4.70%, the average for the original 163 ears. The low-oil strain traces back to ear 110, which when analyzed was found to contain 4.10% oil and 11.13% protein. The average percentage of oil for the stock seed ears was 4.04. Thus, in the 28th year of selection all of the 96 ears of the 4 strains trace back to 4 ears of the original Burr's White.

Even though the ear has been used as the unit of selection and the history of the strains traced through the female side only, the reduction of the ancestry to a single ear for each strain indicates that the strains at the present date are likely to be more nearly homozygous than was the original material from which they came. Further evidence that the strains are more homozygous than open-pollinated varieties is furnished by selfing the strains. It has been shown at this station that upon selfing, a condition of uniformity is reached more quickly than with open-pollinated varieties. Although the individual inbred lines coming from any one of the strains differ among themselves in composition, they are always significantly different from any of the inbreds coming from the other strains.

That there is still some heterozygosity left in the individual within the strains is indicated by the fact that there is a reduction in vigor upon selfing.

East (2), in fitting curves to the data for the first 10 generations of selection, found that at first the curve was concave, showing great progress,

PEDIGREE OF ILLINOIS LOW-PROTEIN CORN

GENERATION NUMBER

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
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*06	10	14	03	06	02	15	09	20	10	04	08		18	10	18	02	08	08	12	08		14	14				13
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PEDIGREE OF ILLINOIS HIGH-PROTEIN CORN

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*These numbers, preceded by generation numbers, denote the seed ear numbers.

PEDIGREE OF ILLINOIS HIGH-OIL CORN

GENERATION NUMBER

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PEDIGREE OF ILLINOIS LOW-OIL CORN

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*These numbers, preceded by generation numbers, denote the seed ear numbers.

later convex, showing that progress became slower, and at last horizontal, indicating that no more progress would result from selection. An inspection of Fig. 1 and 2 presented here will show that, contrary to East's results, there has been considerable progress since the 10th generation for all of the strains with the possible exception of the Illinois low protein. The Illinois high-oil strain has shown greater progress since the 10th generation than it did before.

It does not seem possible to predict a limit to the progress that selection will make in the Illinois high-oil and the Illinois high-protein strains by the application of curves to the data at hand. Apparently the low-protein strain has made but little change in the last 20 years. If the average protein content for the years beginning with 1902 be compared with the protein content of ear 106, to which the strain now traces, no significant difference appears.

The low-oil strain is approaching a physiological limit. The greater percentage of the oil in a grain of corn is contained in the germ (9). Hence, in selection for low-oil content the size of the germ has been decreased both absolutely and relatively in comparison with the size of the endosperm. In the ear containing the lowest percentage of oil on record, namely, 0.69%, 80% of the grains were germless. The necessity of using ears having grains that will germinate naturally tends, therefore, to check the progress of selection, and eventually may stop it altogether.

EFFECT OF SELECTION UPON VARIABILITY

It seems to be accepted by most biologists that selection for a given character in a cross-fertilized crop like corn leads to a lower variability. That this reduction in variability may have its limits is brought out by Davenport (1) when he states that selection in a cross-fertilized crop such as corn simply shifts the type but does not appreciably change the variability unless a physiological limit is reached. As evidence for this belief, he cites Karl Pearson as stating that 10 to 13% reduction in variability obtained by the selection of two parents is almost the limit that can be reached, even if the complete ancestry had been selected. Davenport also uses the first 8 years' data on the Illinois "chemical" strains of corn, employing the coefficient of variation as a measure of variability, and states that there is no significant change in variability. It is of interest to see how the matter stands after the results of 20 more years of continued selection have been secured.

Variability Measured by the Coefficient of Variation

Protein

The effect of selection upon the variability in protein content as measured by the coefficient of variation from the beginning of the experiment through the 28 years of selection can be seen in Fig. 3. Although the yearly variations as measured by this coefficient are rather large, there are decided trends that run in directions opposite to the line of selection. As selection

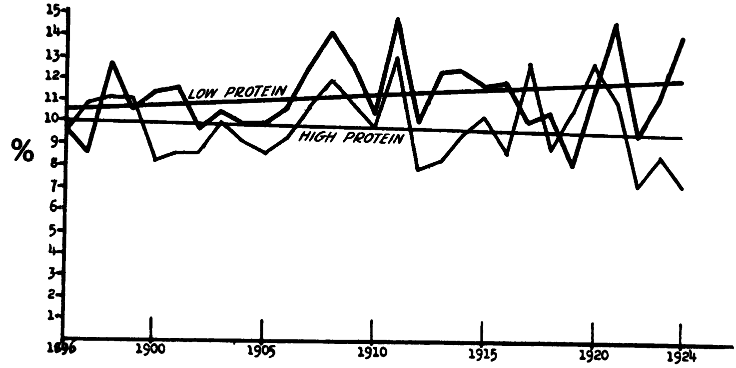

Figure 3. Yearly variations in protein content of corn and their straight-line trends as measured by the coefficient of variation.

leads to a low mean, the variability increases, and vice versa. As measured by the best fitting lines, the variability has increased 14.05% in the low-protein strain over that at the beginning and decreased 4.83% in the high-protein strain.

Oil

Similar results have been obtained for the oil content; only here the divergence is more marked. The variability, according to the coefficient of variability, has increased 69.84% for the low-oil strain and decreased 23.21% for the high-oil strain. (Fig. 4.)

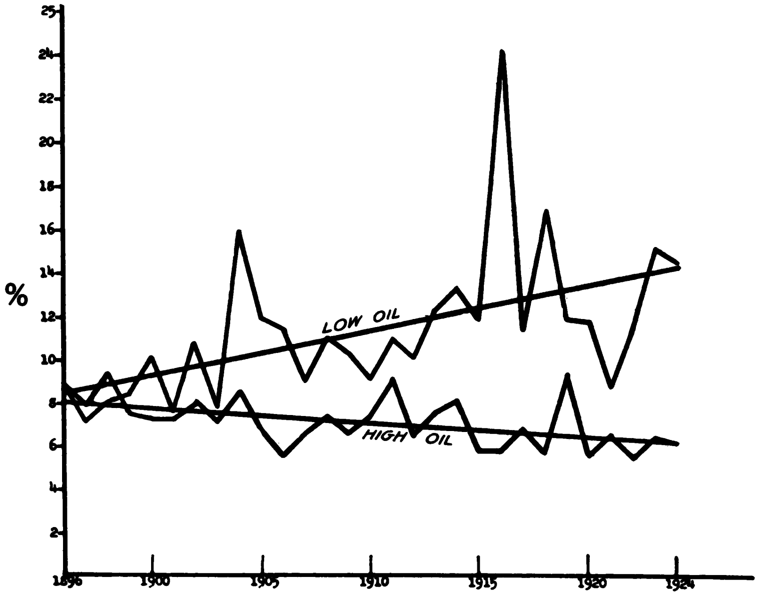

Figure 4. Yearly variations in the oil content of corn and their straight-line trends as measured by the coefficient of variation.

It appears from these results that selection can change variability, as measured by the coefficient of variation, more than 13% and that the variability may either increase or decrease, depending upon the magnitude of the mean. Although the coefficient of variation has been the most commonly used means of comparing variability when different types of variation are involved in the comparison, it is recognized to have decided limitations. Pearl (12) states that, ". . . the coefficient of variation has never been an entirely satisfactory constant to biologists, at least," and also that (11, p. 275) "one should always remember that this constant simply measures the degree of scatter of the distribution in relation to the mean value of the thing varying." Such a relation may have a real and significant meaning but sometimes it does not have, for reasons inherent in the nature of the facts themselves.

Variability Measured by the Standard Deviation

The standard deviation has usually been accepted as the standard method of measuring in absolute terms the degree of variability. It has the advantage that it is a constant of the mathematical formula for the curve of variation representing the distribution of a population.

Protein

The variability for protein content in the Illinois high-protein and Illinois low-protein strains, as measured by the standard deviation (Fig. 5), shows trends opposite to those obtained when the coefficient of variation is used to measure the variation. Although showing considerable yearly fluctu-

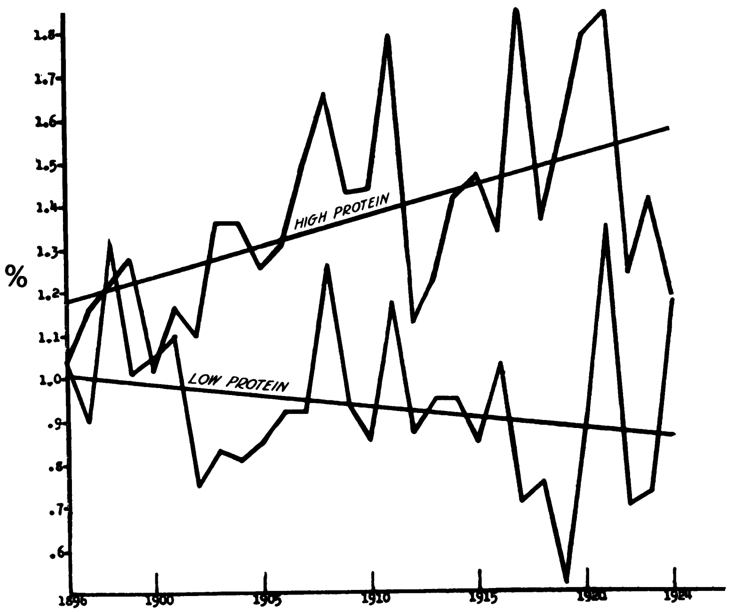

Figure 5. Yearly variations in protein content of corn and their straight-line trends as measured by the standard deviation, 1896-1924.

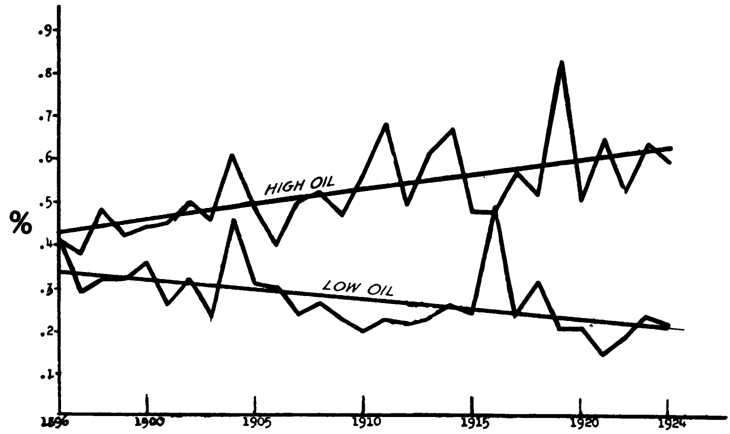

Figure 6. Yearly variations in oil content of corn and their straight-line trends as measured by the standard deviation, 1896-1924.

ation, the variability tends to move in the same direction as the mean; e. g., the high-protein strain is more variable than the low-protein strain for protein content. During the course of the experiments an increase in variability, according to this index, of 32.97% for the high-protein strain and a decrease of 14.71% for the low-protein strain have been obtained.

Oil

The variability measured by the standard deviation for the oil content shows similar results (Fig. 6). Here also the variability is greater in the strain having the higher content. An increase in variability of 50.95% in the high-oil strain and a decrease of 36.44% in the low-oil have been obtained. Again, the greater divergence in variability occurs between the oil strains.

When the standard deviation is used as a measure of variability, greater changes are exhibited than when the coefficient of variation is employed except for the low-oil strain. However, the standard deviation has its limitations also. It can not be used to compare variability except where like things are measured in like units.

Variability Measured by the Weinberg Formula

Weinberg (14) has recently proposed a method for measuring variation which is free from most of the limitations of the coefficient of variation and standard deviation. The coefficient W =

$$\frac{\sigma \ (M_n - M_o)^{1/2}}{[(M_a - M_o)\ (M_n - M_a)]^{1/2}}$$

when M_n is the highest value in the distribution, M_o is the lowest value in the distribution, M_a is the mean value of all variants.

Table 1. Comparison of coefficient of variation with Weinberg's formula for measuring variation.

Example 1		Example 2	
Class	f	Class	f
1	1	11	1
2	1	12	1
3	1	13	1
4	1	14	1
5	1	15	1
6	1	16	1
7	1	17	1
8	1	18	1
9	1	19	1
Mean=5		Mean=15	
S. D. =2.582		S. D. =2.582	
C. V.=51.64%		C. V.=17.21%	
W.=1.83		W.=1.83	

The denominator of the formula measures roughly the skewness of the variability curve, while the second term in the numerator measures the range. The greater the skewness or the wider the range, or both, the greater the variability. The coefficient thus obtained is not affected by the magnitude of the mean as is the coefficient of variation, nor is it limited by unlike material or unlike units of measure as is the standard deviation. Like the coefficient of variation, however, it expresses variation as an abstract figure.

In Table 1 Weinberg's method is compared with the coefficient of variation for measuring variation by means of two examples. In example 1 the mean is 5, the standard deviation, 2.582, and the coefficient of variation is 51.64%. By the Weinberg method the variation is 1.83. In example 2 each class is 10 units higher. The mean is 15, the standard deviation is the same as in example 1, but the coefficient of variation is 17.21%. Variation, as expressed by Weinberg's formula, is the same as in example 1. It is believed that for comparative purposes the Weinberg formula gives a better conception of variability than does the coefficient of variation.

Protein

The effect of selection upon variability for protein content, as measured by the Weinberg formula (Fig. 7), is similar to the results obtained when the standard deviation is used in that variability increases as selection leads to a high mean and decreases as selection leads to a low mean. The increase in variability for the high-protein strain was 25.49%. However, the decrease in variability for the low-protein strain was only 3.94%.

Oil

Similar results were obtained for variability in oil content of the oil strains. (Fig. 8.) The increase in the high-oil strain was 23.97% and the decrease in the low-oil strain was 30.18%. Again, the divergence in variability is greater between the oil strains than between the protein strains.

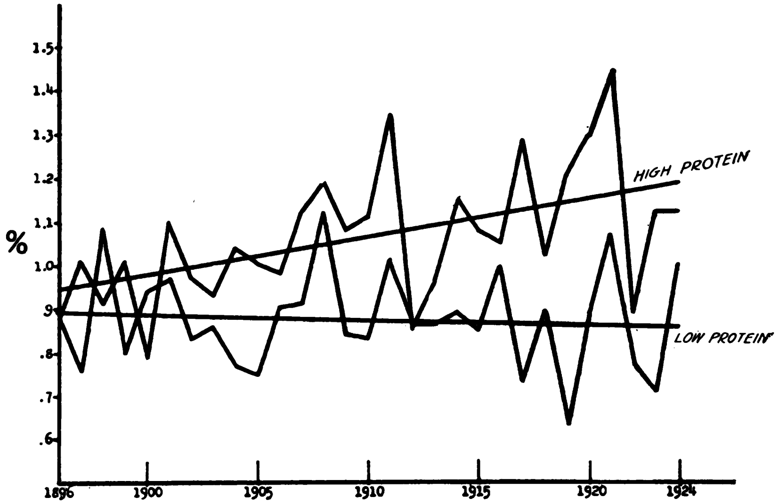

Figure 7. Yearly variations in protein content of corn and their straight-line trends as measured by Weinberg's formula, 1896-1924.

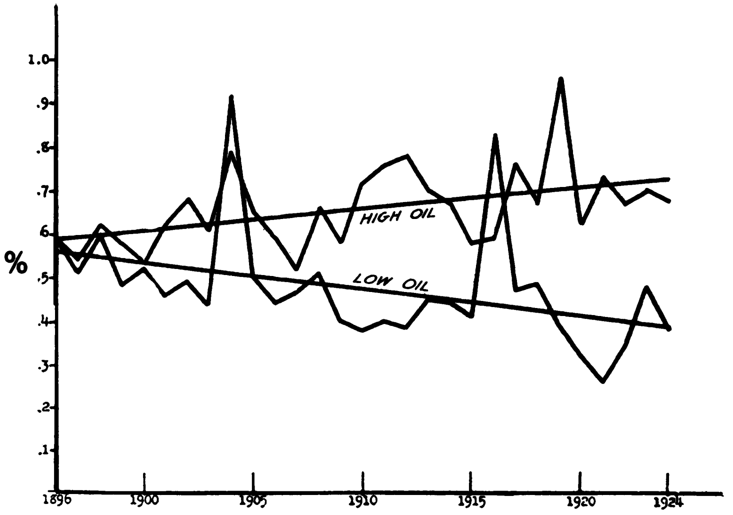

Figure 8. Yearly variations in oil content of corn and their straight-line trends as measured by Weinberg's formula, 1896-1924.

Variability Measured by the Modal Class

Since the type of the population is represented by the modal class, it is of interest to know what proportion of the population resides within the modal class as selection continues.

A graphical representation of the modal classes in respect to composition for the four different strains taken at periodic intervals is seen in Fig. 9.

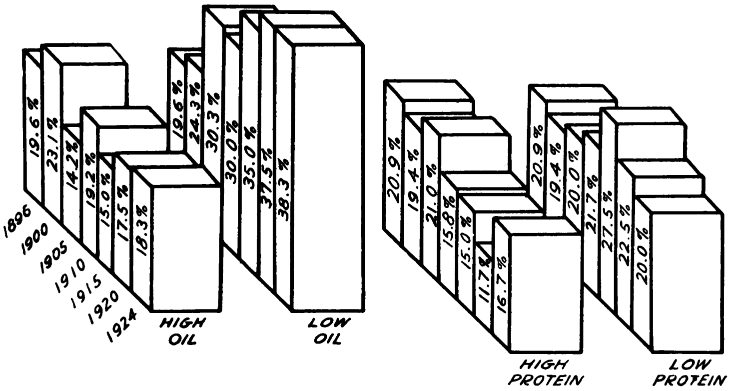

Figure 9. **Graphic presentation of the modal classes with respect to the percentage composition of Illinois high and low oil, and high and low protein corn, at periodic intervals between 1896 and 1924.**

The blocks represent the relative proportion of the population residing in the modal class for the respective years. Although there is considerable fluctuation, it can be seen that the trend is downward for the high-oil and the high-protein strains, i. e., as selection leads toward a higher mean fewer and fewer individuals of the population reside within the modal class. In the low-oil strain there is a decided trend upward. The trend in the low-protein strain is slightly upward. Hence, selection for a low mean increases the percentage of individuals lying in the modal class.

The percentage of the population in the modal class may be taken as a rough measure of the uniformity, and, correspondingly, the percentage outside of the modal class as an expression of variability. In general, the greater the percentage of the population lying in the modal class the less the variability, and vice versa. This variability may be expressed in the form of a ratio $\frac{100-Y}{Y}$ where Y is the percentage of the population lying in the modal class. The smaller Y is the greater the ratio; hence, the greater the variability.

Variability may also be expressed by the inverted straight-line trend for the modal classes. For the purposes of this work such trends were determined by the use of the formulas $\frac{100-Y_o}{Y_o} = X_o$ and $\frac{100-Y_d}{Y_d} = X_d$ when Y_o is the origin and Y_d the destination of the best fitting straight line for the modal classes, and X_o and X_d are the origin and destination, respectively, for the lines to be determined. The quotient thus obtained may be called the extramodal coefficient because it takes into account the population outside the modal class. The trends thus obtained (Figs. 10, 11, 12, 13) indicate that variability becomes less as a low mean is approached and greater as a high mean is approached. The method is empirical and therefore has the limitations that most empirical formulas have.

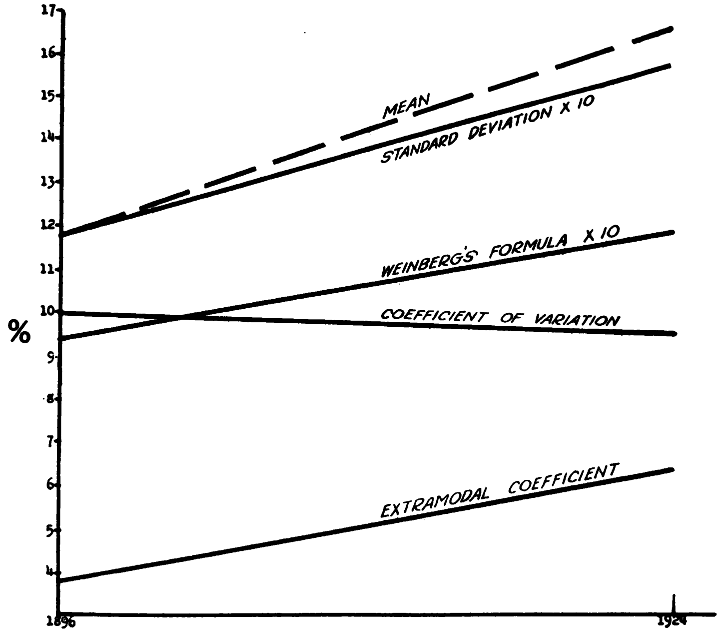

Figure 10. Comparison of four different measures of variation for protein content of Illinois high-protein corn as expressed by the best fitting straight lines; the broken line shows the trend of the mean protein content.

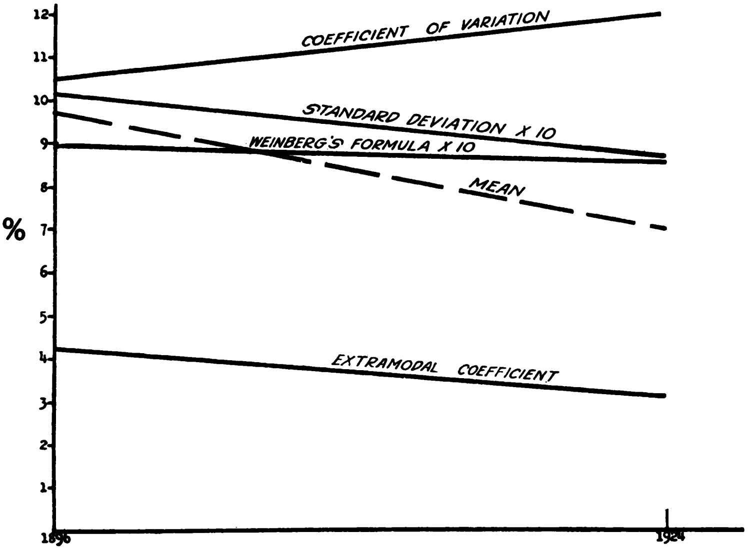

Figure 11. Comparison of four different measures of variation for protein content of Illinois low protein corn as expressed by the best fitting straight lines; the broken line shows the trend of the mean protein content.

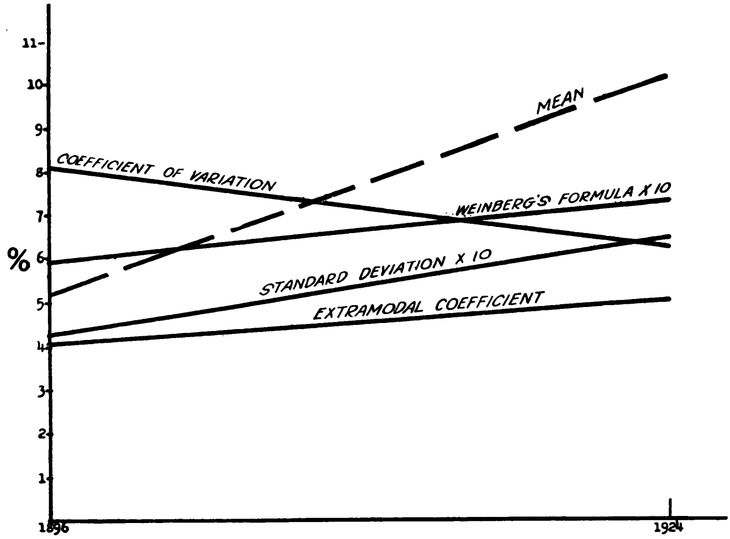

Figure 12. Comparison of four different measures of variation for oil content of Illinois high-oil corn as expressed by the best fitting lines; the broken line shows the trend of the mean oil content.

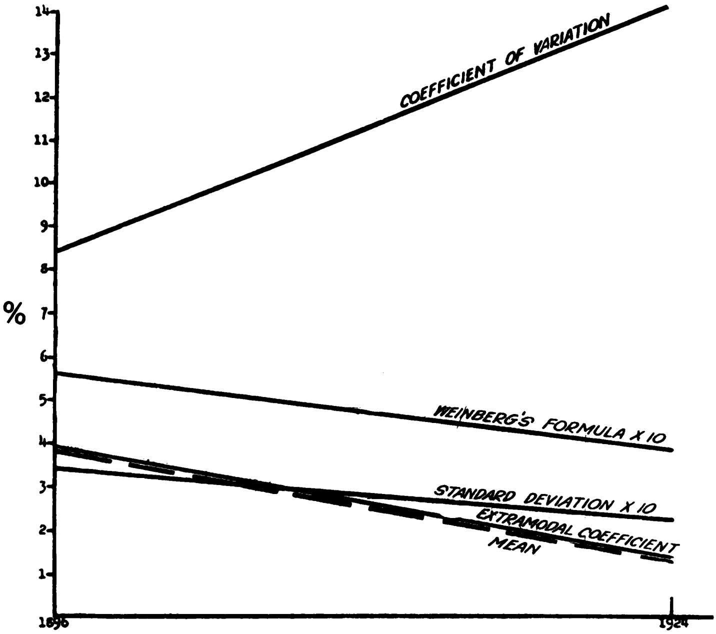

Figure 13. Comparison of four different measures of variation for oil content of Illinois low-oil corn as expressed by the best fitting straight lines; the broken line shows the trend of the mean oil content.

Table 2. Comparison of change in variability for chemical composition in four Illinois strains of corn as determined by the best fitting lines for four different measures of variability; 1896 and 1924.

Strain	Method used to measure variability	Year		Percentage of increase (+) or decrease (−)
		1896	1924	
High protein	Coefficient of variation	10.03	9.54	[a] −4.86
	Standard deviation	1.18	1.57	+33.05
	Weinberg's formula	.95	1.19	+25.26
	Extramodal coefficient	3.79	6.37	+68.07
Low protein	Coefficient of variation	10.53	12.02	+14.16
	Standard deviation	1.01	.86	−14.85
	Weinberg's formula	.89	.86	−3.37
	Extramodal coefficient	4.19	3.14	−25.06
High oil	Coefficient of variation	8.11	6.23	−23.18
	Standard deviation	.43	.64	+48.84
	Weinberg's formula	.59	.73	+23.73
	Extramodal coefficient	4.09	5.02	+22.74
Low oil	Coefficient of variation	8.50	14.44	+69.88
	Standard deviation	.34	.21	−38.24
	Weinberg's formula	.56	.39	−30.36
	Extramodal coefficient	3.68	1.50	−59.24

[a] These figures differ slightly from those in the text. The figures in the text were based on four figures after the decimal.

COMPARISON OF THE DIFFERENT MEASURES OF VARIABILITY

A comparison of the four different measures of variability discussed above for each of the four strains is shown in Figs. 10, 11, 12, and 13. The standard deviation and the coefficient obtained by the Weinberg method are multiplied by 10 so that they may be plotted on a common scale with the other measures. It is to be noted that in all cases variability progresses in the same direction as the mean when measured by the standard deviation, Weinberg formula, and the extramodal coefficient. If variability is expressed as a percentage of the mean it decreases as the mean increases, and vice versa. Of the four strains, the low protein shows on an average the least change in variability (Table 2).

RELATIVE VARIABILITY IN PROTEIN AND OIL

The protein strains show greater variability than do the oil strains (Tables 3 to 6), thus suggesting that the former may be affected to a greater extent by the environment, Hopkins states (8, p. 239) "the fat content of corn is even more susceptible to the influence of seed selection than is the protein content, doubtless due to the fact that the primary materials from which fat is manufactured, namely, carbon dioxide and water, are usually furnished to the plant in unlimited supply, while the formation of protein is essentially dependent upon the supply of available nitrogen in the soil." Variability is usually augmented when the living material under study is grown at a minimum or maximum rather than at the optimum condition.

Table 3. Average protein content and its percentage variability in Illinois high-protein strain corn, 1896-1924, as measured by different methods, together with extreme variates.

Year	Ears analyzed	Average protein content	Standard deviation	Coefficient of variation	Variability by Weinberg's formula	Lowest variate	Highest variate	Population in modal class
	Number	*Per cent*			*Per cent*			*Per cent*
1896	163	[a] 10.93	[a] 1.04	[a] 9.50	0.881	8.3	13.9	20.9
1897	112	10.99	1.16	10.90	1.008	8.3	13.6	----------
1898	252	10.98	1.22	11.15	.912	7.7	14.9	----------
1899	216	11.62	1.28	11.00	1.012	8.4	14.8	----------
1900	216	12.62	1.02	8.09	.795	9.3	15.7	19.4
1901	114	13.78	1.17	8.48	1.103	11.5	16.0	----------
1902	90	12.90	1.10	8.50	.965	9.5	15.0	----------
1903	100	13.51	1.36	10.04	.925	8.5	17.3	----------
1904	100	15.03	1.36	9.05	1.040	10.6	17.8	----------
1905	119	14.73	1.26	8.55	.998	10.8	17.4	21.0
1906	120	14.26	1.31	9.19	.978	10.5	17.7	----------
1907	120	13.90	1.49	10.72	1.119	10.3	17.4	----------
1908	119	13.94	1.66	11.91	1.193	9.4	17.3	----------
1909	120	13.29	1.43	10.76	1.076	9.2	16.4	----------
1910	120	14.87	1.44	9.68	1.109	11.2	18.0	15.8
1911	120	13.79	1.79	12.98	1.344	10.3	17.4	----------
1912	120	14.49	1.13	7.80	.854	10.3	17.5	----------
1913	120	14.83	1.22	8.23	.965	11.6	18.0	----------
1914	120	15.04	1.42	9.44	1.148	11.5	17.8	----------
1915	120	14.54	1.47	10.19	1.076	10.7	18.2	15.0
1916	120	15.66	1.34	8.56	1.047	12.7	19.3	----------
1917	120	14.45	1.85	12.80	1.277	10.2	18.6	----------
1918	120	15.49	1.36	8.78	1.022	11.8	18.9	----------
1919	120	14.70	1.55	10.54	1.201	11.1	17.8	----------
1920	120	14.01	1.79	12.78	1.287	9.5	17.4	11.7
1921	120	16.66	1.84	11.04	1.441	9.4	18.8	----------
1922	120	17.34	1.24	7.15	.890	12.6	20.6	----------
1923	120	16.53	1.41	8.50	1.116	13.1	19.7	----------
1924	120	16.60	1.19	7.17	1.120	14.6	19.2	16.7

[a] Data for the years 1896–1903 are taken from Davenport's Principles of Breeding (*1, p. 446*) means and standard deviations for the remaining years are calculated by the nongrouping method.

Table 4. Average protein content and its percentage variability in Illinois low-protein strain corn, 1896-1924, as measured by different methods, together with extreme variates.

Year	Ears analyzed	Average protein content	Standard deviation	Coefficient of variation	Variability by Weinberg's formula	Lowest variate	Highest variate	Population in modal class
	Number	*Per cent*			*Per cent*			*Per cent*
1896	163	[a] 10.93	[a] 1.04	[a] 9.50	0.881	8.3	13.9	20.9
1897	60	10.63	.90	8.47	.759	8.2	14.0	----------
1898	126	10.49	1.32	12.61	1.089	7.5	13.4	----------
1899	144	9.59	1.01	10.50	.802	6.7	13.1	----------
1900	144	9.13	1.04	11.34	.937	7.1	12.3	19.4
1901	126	9.63	1.10	11.47	.975	7.6	13.1	----------
1902	90	7.86	.75	9.60	.829	6.4	9.7	----------
1903	100	8.00	.83	10.41	.862	6.4	10.2	----------
1904	100	8.17	.81	9.91	.773	6.1	10.5	----------
1905	120	8.58	.85	9.91	.753	6.6	12.1	20.0
1906	120	8.65	.92	10.64	.899	6.7	10.9	----------
1907	120	7.32	.92	12.57	.910	5.8	10.5	----------
1908	120	8.96	1.26	14.06	1.117	6.3	11.4	----------
1909	120	7.48	.94	12.57	.842	5.5	10.8	----------
1910	120	8.26	.86	10.41	.827	6.5	11.0	21.7
1911	120	7.90	1.17	14.81	1.005	5.9	12.1	----------
1912	120	8.23	.82	9.96	8.860	6.8	10.8	----------
1913	120	7.71	.95	12.32	.861	5.7	10.8	----------
1914	120	7.67	.95	12.39	.890	5.9	10.8	----------
1915	120	7.27	.85	11.69	.854	5.7	9.9	27.5
1916	120	8.68	1.03	11.86	.994	6.6	10.9	----------
1917	120	7.09	.71	10.01	.233	5.6	9.6	----------
1918	120	7.13	.75	10.52	.894	5.9	8.8	----------
1919	120	6.46	.52	8.05	.629	5.4	8.3	----------
1920	120	7.54	.89	11.80	.890	6.0	10.5	22.5
1921	120	9.14	1.35	14.77	1.074	6.6	13.4	----------
1922	120	7.42	.70	9.43	.774	6.1	9.6	----------
1923	120	6.48	.73	11.27	.708	5.0	9.4	----------
1924	120	8.38	1.17	13.96	.998	6.1	11.8	20.0

[a] Data for the years 1896, 1898, 1899, 1900, 1901, 1902, and 1903 are taken from Davenport's Principles of Breeding (*1, p. 446*); means and standard deviations for the remaining years are calculated by the nongrouping method.

Table 5. Average oil content and its percentage variability in Illinois high-oil strain corn, 1896-1924, as measured by different methods, together with extreme variates.

Year	Ears analyzed	Average oil content	Standard deviation	Coefficient of variation	Variability by Weinberg's formula	Lowest variate	Highest variate	Population in modal class
	Number	*Per cent*			*Per cent*			*Per cent*
1896	163	[a] 4.68	[a] 0.41	[a] 8.83	0.585	3.9	6.0	19.6
1897	80	4.79	.38	7.87	.543	3.6	5.7	---------
1898	216	5.10	.48	9.33	.615	4.1	6.7	---------
1899	108	5.65	.42	7.47	.582	4.3	6.5	---------
1900	108	6.10	.44	7.26	.527	4.6	7.4	23.1
1901	126	6.24	.45	7.26	.621	4.9	7.1	---------
1902	90	6.25	.50	8.06	.680	5.0	7.2	---------
1903	100	6.51	.46	7.07	.608	5.5	7.6	---------
1904	101	7.11	.61	8.61	.792	6.0	8.4	---------
1905	120	7.30	.49	6.70	.651	6.3	8.6	14.2
1906	120	7.38	.40	5.46	.594	6.6	8.5	---------
1907	120	7.43	.51	6.82	.522	6.2	8.7	---------
1908	120	7.21	.53	7.36	.658	5.9	8.5	---------
1909	120	7.05	.47	6.62	.580	5.8	8.4	---------
1910	120	7.72	.57	7.36	.718	6.5	9.0	19.2
1911	120	7.52	.69	9.20	.763	5.9	9.2	---------
1912	120	7.71	.50	6.49	.778	6.5	8.7	---------
1913	120	8.16	.62	7.63	.700	6.4	9.6	---------
1914	120	8.30	.68	8.23	.668	5.8	10.1	---------
1915	120	8.47	.49	5.77	.576	6.9	9.8	15.0
1916	120	8.51	.49	5.80	.592	7.2	10.0	---------
1917	120	8.52	.58	6.78	.762	7.2	9.7	---------
1918	120	9.36	.53	5.67	.674	8.0	10.5	---------
1919	120	9.06	.84	9.25	.961	7.3	10.4	---------
1920	120	9.28	.52	5.57	.619	7.8	10.6	17.5
1921	120	9.94	.66	6.64	.729	8.4	11.7	---------
1922	120	9.86	.54	5.48	.674	8.7	11.3	---------
1923	120	10.08	.65	6.45	.695	8.3	11.8	---------
1924	120	9.86	.61	6.19	.676	8.4	11.7	18.3

[a] Data for the years 1896–1903 are taken from Davenport's principles of breeding (*1, p. 446*); means and standard deviations for the remaining years are calculated by the nongrouping method.

Table 6. Average oil content and its percentage variability in Illinois low-oil strain corn, 1896-1924, as measured by different methods, together with extreme variates.

Year	Ears analyzed	Average oil content	Standard deviation	Coefficient of variation	Variability by Weinberg's formula	Lowest variate	Highest variate	Population in modal class
	Number	*Per cent*			*Per cent*			*Per cent*
1896	163	[a] 4.68	[a] 0.41	[a] 8.83	0.585	3.9	6.0	19.6
1897	50	4.10	.29	7.10	.510	3.4	4.7	---------
1898	108	3.59	.32	8.13	.589	3.2	4.8	---------
1899	144	3.85	.32	8.42	.484	2.8	4.6	---------
1900	144	3.57	.36	10.13	.522	2.6	4.5	24.4
1901	126	3.45	.26	7.59	.456	2.8	4.1	---------
1902	90	3.00	.32	10.83	.492	2.1	3.8	---------
1903	90	2.99	.23	7.83	.441	2.5	3.6	---------
1904	100	2.89	.46	15.91	.920	2.4	3.4	---------
1905	119	2.58	.31	11.86	.502	1.8	3.1	30.3
1906	120	2.67	.30	11.35	.443	1.6	3.5	---------
1907	120	2.60	.24	9.04	.466	2.2	3.3	---------
1908	120	2.39	.27	11.09	.507	1.8	2.9	---------
1909	120	2.24	.23	10.40	.402	1.4	2.8	---------
1910	120	2.21	.20	9.05	.384	1.6	2.7	30.0
1911	120	2.06	.23	11.02	.398	1.4	2.7	---------
1912	120	2.19	.22	10.05	.386	1.3	2.7	---------
1913	120	1.91	.24	12.30	.451	1.3	2.4	---------
1914	120	1.98	.26	13.33	.446	1.3	2.7	---------
1915	120	2.07	.25	11.93	.410	1.4	3.1	35.0
1916	120	2.07	.50	24.30	.828	1.3	4.7	---------
1917	120	2.10	.24	11.38	.474	1.7	2.8	---------
1918	120	1.88	.32	16.86	.489	1.2	3.0	---------
1919	120	1.77	.21	11.92	.394	1.3	2.5	---------
1920	120	1.80	.21	11.83	.323	1.0	2.4	37.5
1921	120	1.71	.15	8.77	.264	1.0	2.3	---------
1922	120	1.68	.19	11.55	.347	.9	2.2	---------
1923	120	1.58	.24	15.19	.480	1.1	2.1	---------
1924	120	1.51	.22	14.57	.387	.9	2.2	38.3

[a] Data for the years 1896, 1898, 1899, 1900, 1901, 1902, and 1903 are taken from Davenport's Principles of Breeding (*1, p. 446*); means and standard deviations for the remaining years are calculated by the nongrouping method.

SYMMETRY OF DISTRIBUTION

A number of other statistical expressions have been proposed intended to describe the nature of a population with respect to its distribution. Certain of these expressions are of interest in connection with the present study. An important item of information in regard to a distribution is whether the variates are symmetrically distributed with reference to the mean or whether there is a bunching of variates on one side of the mean and a long tailing out of the variates on the other side; i. e., to know the amount of skewness.

Another item that should be known is whether the variates are densely grouped at the mean, giving a high peak to the frequency polygon, or whether the distribution is rather flat in the middle and contracted at the ends, or whether the distribution of the variates is intermediate between these two conditions; i. e., to know the amount of kurtosis. A normal distribution is said to be mesokurtic, a peaked curve leptokurtic, and a flat curve platykurtic.

The mean, median, mode, standard deviation, coefficient of variation, variation by Weinberg's formula, skewness, and kurtosis are given in Table 7 for each of the four strains taken at periodic intervals. The median, mode, skewness, and kurtosis were calculated on the basis of percentiles.[1] Kelley (10, p. 58–62, 75–77) states that "this method of determining curve types, although in general not as accurate as the longer method of Pearson, can be used where the populations are large and the standard errors are small." The variates of the high-oil and the low-oil strains appear to be fairly symmetrically distributed with reference to the mean. In no case is skewness significantly different from zero. In 5 of the 7 years studied the median is greater than the mean in the high-oil strain. In the low-oil strain the median is lower than the mean in 4 of the 7 years. The differences in all cases are very small.

The distributions for the low-protein strain are negatively skewed, i. e., tail out on the high side for each of the years studied except 1896. Although the skewness is much greater than in the case of the oil strains, in no instance is skewness significantly different from zero. Likewise, the distributions for the high-protein strain show a greater skewness than do the distributions for the oil strains, but in no case is the skewness significantly different from zero.

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[1] Mode = Mean – 3.03 (mean – median); Skewness = $P_{.50} - \frac{1}{2}D$ (S.D. of Sk = $0.55914\ D/N^{1/2}$, $D = P_{.50} - P_{.10}$); Kurtosis = Q/D (Q = quartile deviation, S.D. of Ku = $0.27779/N^{1/2}$). To determine the class in which the $pN + \frac{1}{2}$ measure lies, let f_p = the frequency in this class, i_p = the interval or range covered by this class, F_p = the sum of the frequencies in all classes below this class, v_p = the value of the lower boundary of this class, N = the total population, P_p = percentile (the value of which is to be calculated), p = proportion of classes having values smaller than P_p. Then:

$$P_p = v_p + [(pN - F_p)/f_p]\, i_p.$$

Table 7. Measurements of central or average tendencies, and of dispersions for distributions of the variates of four Illinois strains of corn taken at periodic intervals.

HIGH-PROTEIN STRAIN

Year	Number of ears analyzed	Mean	Median	Mode	Standard deviation [a]	Coefficient of variation [a]	Variability by Weinberg's formula [a]	Skewness [b]	Kurtosis [c]
1896	163	10.96	11.04	11.18	1.04	9.50	0.88	+0.1082±0.0874	−0.0008±0.0147
1900	216	12.69	12.64	12.55	1.02	8.09	.80	−.1312± .0704	+.0101± .0127
1905	119	14.77	14.94	15.29	1.26	8.55	1.00	+.3997± .2312	−.0320± .0172
1910	120	14.93	15.08	15.37	1.44	9.68	1.11	+.2159± .1313	+.0229± .0171
1915	120	14.55	14.67	14.92	1.47	10.19	1.08	+.2447± .1426	+.0106± .0171
1920	120	14.04	13.92	13.67	1.79	12.78	1.29	−.2885± .1775	+.0206± .0171
1924	120	16.68	16.65	16.58	1.19	7.17	1.12	−.1027± .1237	−.0065± .0171
LOW-PROTEIN STRAIN									
1896	163	10.96	11.04	11.18	1.04	9.50	0.88	+0.1082±0.0874	+0.0008±0.0147
1900	144	9.19	9.07	8.83	1.04	11.34	.94	−.2044± .0922	+.0043± .0156
1905	120	8.64	8.48	8.15	.85	9.91	.75	−.1772± .0946	+.0398± .0171
1910	120	8.32	8.24	8.08	.86	10.41	.83	−.1443± .0870	−.0085± .0171
1915	120	7.30	7.16	6.85	.85	11.69	.85	−.2182± .0775	+.0018± .0171
1920	120	7.60	7.50	7.29	.89	11.80	.89	−.1543± .0835	+.0061± .0171
1924	120	8.44	8.34	8.14	1.17	13.96	1.00	−.1865± .1049	−.0050± .0171
HIGH-OIL STRAIN									
1896	163	4.74	4.83	5.00	0.41	8.83	0.59	+0.0323±0.0391	−0.0368±0.0147
1900	108	6.15	6.20	6.31	.44	7.26	.53	+.0654± .0653	−.0051± .0180
1905	120	7.35	7.34	7.32	.49	6.70	.65	−.0053± .0519	+.0330± .0171
1910	120	7.78	7.72	7.60	.57	7.36	.72	−.1090± .0507	−.0274± .0171
1915	120	8.52	8.58	8.69	.49	5.77	.58	+.1098± .0574	+.0014+ .0171
1920	120	9.34	9.35	9.38	.52	5.57	.62	+.0850± .0491	−.0192± .0171
1924	120	9.92	10.00	10.17	.61	6.19	.68	+.0117± .0726	−.0301± .0171
LOW-OIL STRAIN									
1896	163	4.74	4.83	5.00	0.41	8.83	0.59	+0.0323±0.0391	−0.0368±0.0147
1900	144	3.63	3.61	3.56	.36	10.13	.52	−.0565+ .0304	+.0007± .0152
1905	119	2.64	2.62	2.58	.31	11.86	.50	−.0197± .0262	+.0250± .0172
1910	120	2.26	2.27	2.29	.20	9.05	.38	+.0260+ .0241	+.0171± .0171
1915	120	2.13	2.12	2.12	.25	11.93	.41	−.0177± .0209	+.0155± .0171
1920	120	1.84	1.86	1.89	.21	11.83	.32	+.0257± .0227	−.0268± .0171
1924	120	1.56	1.54	1.52	.22	14.57	.39	−.0130± .0240	−.0322± .0171

[a] Taken from Tables 3, 4, 5, and 6.

[b] A positive sign (+) indicates that the distribution tails out on the low side.; a negative sign (−) indicates the reverse.

[c] Data reported equals Ku. less 0.26315. If sign is positive (+) curve is platykurtic; if sign is negative (−) curve is leptokurtic.

The four strains show but little deviation from a mesokurtic curve. The small deviations from the normal probability curve are as frequent in the direction of a leptokurtic curve as of a platykurtic curve.

A study of the extreme variates of the different strains for the period of selection (Fig. 14) shows that in the high-oil strain the variates deviate as far on the low side as they do on the high side. In the low-oil strain the deviation is slightly greater on the high side. However, the extreme variates in the high-oil strain are about twice as far from the mean as those in the low-oil strain. The range in the low-oil strain is becoming less while that in the high-oil strain is becoming greater.

The extreme variates in the low-protein strain are farther from the mean in a positive direction than in a negative direction, while in the high-

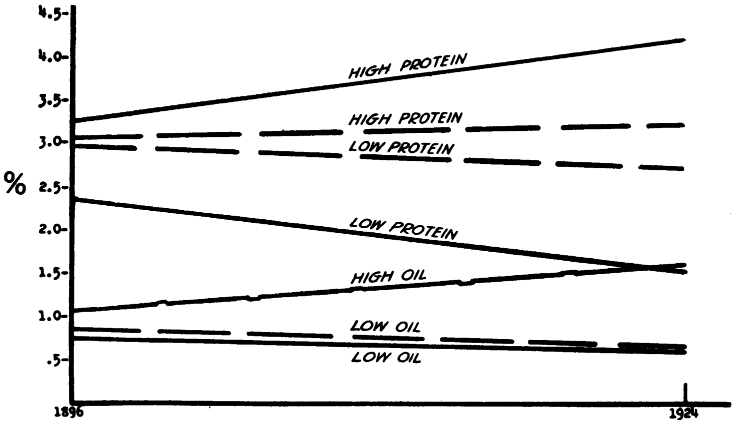

Figure 14. Comparison of the range between the lowest variate and the mean (solid line), and the highest variate and the mean (broken line), for high-protein, low-protein, high-oil, and low-oil Illinois corn as expressed by the best fitting straight lines.

protein strain the reverse is true. This means that the distribution tails out toward the mean of the original nonselected material in both cases.

GENERAL DISCUSSION

If, as has been stated by many, the population with the greatest variability offers the greatest chance for improvement by selection then it should be possible to make still greater progress in the Illinois high-protein and Illinois high-oil strains in future years than has been made in the past because the range of each was greater in 1924 than it was in 1896 (Fig. 15). Such a conclusion would be rather questionable. As has been stated, the strains now appear to be more nearly homozygous than was the original material, and our knowledge of genetics and the effects of selection would lead us to believe that they should be. Being more nearly homozygous, they should be less variable genetically. The apparent increase in variability must then be due either to the environment or to the methods of measuring variability.

Emerson (4, p. 30—31) in speaking of the variability in number of rows on an ear of corn says: ". . . It is more reasonable to suppose that an ear which can vary in any one of eight spikes will show a greater degree of fluctuation than one which can vary only in any one of four spikes. For this reason it is likely that strains with a high number of rows will never show the low variability seen in strains with a low number of rows." One may reason in like manner about variability of the oil and of the protein content for the different strains. The high strains are more variable than the low strains because there is more material present for the environment to interact with. Supposing the germ of the high-oil strain to contain 600 cells and that of the low oil to contain 300 cells, we might expect twice the effect on the high-oil

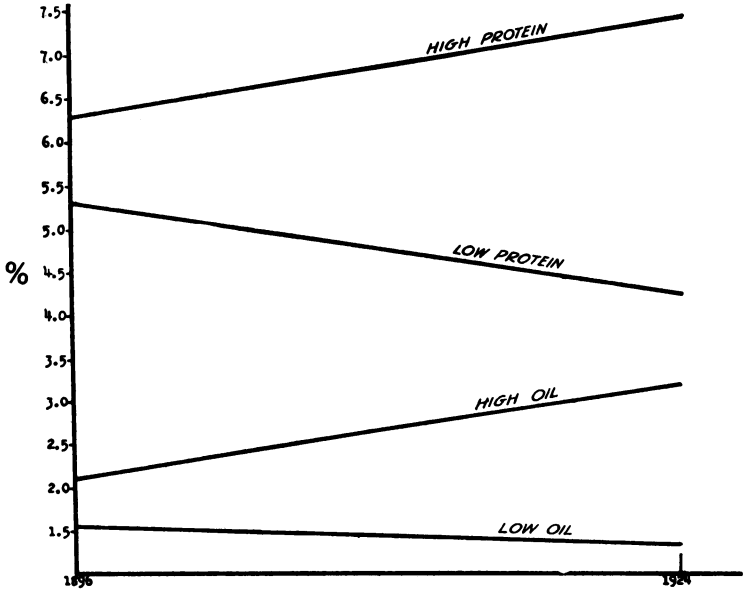

Figure 15. Comparison of the ranges between the highest variate and the lowest variate for high-protein, low-protein, high-oil, and low-oil Illinois corn as expressed by the best fitting straight lines.

strain from environmental action as on the low oil under identical conditions.

Zeleny (15, p. 15) states: "It is a common principle of embryology that a changed condition does not act by accretion, i. e., by addition or subtraction of individual parts without affecting the rest. On the contrary, the action is upon all of the preexisting parts of the organ." This he calls the theory of proportionate action. Zeleny, in the study of the effect of selection for eye facet number in the white bar-eye race of *Drosophila melanogaster,* found that a race with 300 facets was affected ten times as much as a race with only 30 facets by a 1C change in temperature. In order to measure the variability of races with different facet numbers he scaled his classes for grouping so that each class range was a fixed percentage of the mean of its class. He used as a mid or zero point the mean of the original population from which his selected material came. He (15, p. 15) goes on to explain that "the standard deviation, as determined by this method, is expressed in factorial units and serves directly as a coefficient of variation strictly comparable in all cases, regardless of the mean values of the different stocks that may be compared."

Should such a method of measuring variability be applied to the distributions of the oil and protein strains in the present study, it can be readily seen that the degree of deviation from the means would depend upon the value taken as a percentage of the means of the classes. The larger this figure the smaller the deviation of the high strain as compared to the low strain. Also, the farther the high strain is removed from the low strain the greater will be its comparative reduction in variability. The factorial method of measuring variability has not been used in an analysis of these data because the method affords no manner of determining the figure to be used in arriving at the class ranges with such data. Such a figure might be 10, 20, or 23%. Zeleny apparently uses such a figure as will give a normal distribution for the populations studied. As has been shown, the distributions for protein and for oil content thus far studied do not show a significant deviation from the normal curve.

Although it appears logical to attribute the increase in variation in the high strains to the fact that there is more material for the environment to interact with, it is impossible to prove it, because the variability due to the environment can not be separated from the variability due to the segregation and recombination of factors. The complexity of the inheritance of protein and oil content and the manner of conducting the selection work make it entirely impossible to analyze the four strains genetically. Others who have studied the protein content of corn have also found it impossible to determine the genetic factors involved. East (3) lists a large number of factors other than genetic that may and probably do affect protein content, e. g., number of seeds on the ear, size of pericarp, lack of phosphorus, and departure from optimum temperature and moisture at critical periods. In addition to these there may be considered the factors affecting size of germ, size of endosperm, absorption of different amounts of food elements through the roots and their translocation through the stalk and subsequent deposition within the pericarp. The total protein content is made up chemically of at least four protein groups (13) each of which may be represented by a single or several genes in the germ plasm. Some of these factors may be dominant and others recessive, or there may be any graduation of dominance. Hayes (5) states: "Protein content is therefore inherited in much the same way as other characters which are dependent for their full expression on many different inherited factors of the plant and likewise upon environmental conditions." What has been said of the protein content may likewise be said of the oil content.

SUMMARY

Twenty-eight years of continuous selection for protein and oil content in corn has produced four types which are distinctly different in their composition. When compared with the original nonselected material the high-protein and the high-oil strains show a proportional increase of 50.01 and 109.79%, respectively. The low-protein and low-oil strains show a proportional decrease of 23.26 and 67.87%, respectively.

The high-protein and high-oil strains show no indications of having reached a limit to further increases.

The low-protein strain has changed but little during the last 20 years.

The low-oil strain is approaching a physiological limit to further decreases. Ears with extremely low oil content have a high percentage of germless seeds.

The four different strains now trace back in their pedigrees to a single ear each.

Variability has been shown to change considerably following selection. The degree of change in variability depends somewhat upon the method used in measuring it.

Variability of oil or protein content appears to depend upon the magnitude of the mean of the selected character.

Variability as measured by the coefficient of variation increases when selection leads to a low mean, and vice versa.

Variability, as measured by the standard deviation, Weinberg's formula, and extramodal coefficient, increases when selection leads to a high mean, and vice versa.

The percentage of the population lying in the modal classes decreases when selection leads to a high mean, and vice versa.

The symmetry of the distribution curve as determined by the percentile method for the four strains taken at periodic intervals is not significantly different from that of the normal variability curve.

It is suggested that the apparent increase in variability of the high strains may be due to the fact that there is more material present for the environment to interact with.

ACKNOWLEDGMENT

The writer wishes to thank Dr. L. H. Smith for suggesting the problem. To Dr. C. M. Woodworth, under whose direction these studies were made, the writer expresses sincere thanks for suggestions for analyzing the data and for valuable assistance in the preparation of this paper.

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5

Fifty Generations of Selection for Protein and Oil in Corn

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R. W. JUGENHEIMER

INTRODUCTION

Fifty generations of selection at the Illinois Station for high and low protein and high and low oil in corn were completed with the 1949 crop. This experiment was started in 1896 in the Burr White variety by Dr. Cyril G. Hopkins. It was continued to 1921 by Dr. L. H. Smith and his co-workers. Direction of the project was assumed by the senior author in 1921 and continued to the present time with the help of various persons who have been on the Plant Breeding staff since that time. The work was suspended for 3 years, 1942, 1943, and 1944, because of lack of manpower during the war. However, seed of the 1941 crop was held over and used to plant the 1945 crop.

Few adequate reports of progress on this experiment have been published especially during the latter part of the 50-generation period. The first report was published by Hopkins (3) in 1899 after 3 generations; the second by Smith (4) in 1908, after 10 generations; and the third by Winter (6) in 1929, after 28 generations. Progress on generations 29 to 50 inclusive has been described in the annual reports of the Illinois Agricultural Experiment Station. Charts showing progress of selection for generations 1 to 47 inclusive were presented by Woodworth and Jugenheimer (7).

The purpose of this paper is to give in brief form the main results and trends of selection for protein and oil in corn for 50 generations together with observations on variability of the selected strains and effect of non-random selection and of reverse selection. It is planned to publish a complete report as an Illinois Experiment Station bulletin at a later time.

EXPERIMENTAL PROCEDURE

Selection was begun in 1896 from a foundation seed stock of 163 ears of the Burr White variety of corn. Four selected strains were established, namely: Illinois High Oil, Low Oil, High Protein, and Low Protein. Ear-to-row selection was practiced for the first 28 generations of the experiment, with each selected strain being grown in a separate isolated plot. Within each plot, after the first nine generations of selection, alternate rows were detasseled and seed was saved only from the highest yielding detasseled rows. After 1921 (25 generations) yielding ability was disregarded and ears were saved for chemical analysis from all 12 of the detasseled rows. This changed procedure was continued until 1925.

The number of ears analyzed in each strain was somewhat variable during the first nine generations of the experiment. In the tenth to twenty-eighth generations of selection, 120 ears of each strain were harvested for analysis. The 24 ears most extreme in the desired direction of selection were used as seed for the succeeding generation. For example, in Illinois High Oil 120 ears were selected for each year for oil analysis from the six highest yielding detasseled rows, or 20 ears from each row. From 1921 to 1925, 10 ears were taken for analysis from each of the 12 detasseled rows. The 24 ears with the highest oil content were then used as seed for planting the High Oil plot the next year.

In 1925, the breeding system and method of selection were considerably altered, because of the difficulty of locating isolated plots. Ear-to-row selection was discontinued, and the number of ears harvested from each strain was reduced to 60. In each strain, the 12 ears most extreme in the desired direction of oil or protein content were saved for seed. These were divided into two bulked lots of seed from six ears each, and the lots were planted separately in adjoining plots. Pollinations were made by hand, using bulk pollen mixtures from one lot of a given strain. on silks of the other lot, and *vice versa*. At harvest, 30 ears of each lot were saved for analysis. This procedure has been followed without change up to the present time.

All chemical analyses reported in this paper are on a moisture-free basis. Sampling methods used were discussed by Smith (4). Detailed descriptions of the analytical methods used in the earlier stages of the experiment were given by Hopkins (2). The analytical methods followed more recently have been summarized in part by Hoener and DeTurk (1). At present, total nitrogen is determined by the official Kjeldahl—Gunning—Arnold method, except that the mercury or mercuric oxide was replaced by approximately 0.15 g copper wire. Protein percentage is found by multiplying total nitrogen percentage by 6.25. Oil percentage was determined by extraction with Skellysolve F for 16 hours in a Butt apparatus.

RESULTS

Selection for Oil Content

The mean oil content of the foundation seed lot of Burr White was 4.70% in 1896. In 1949, after 50 generations of selection, the mean oil content of High Oil was 15.36%, while that of Low Oil was 1.01% (Table 1). The range between the means of the two strains was thus 14.35%, the greatest observed during the entire experiment. The mean oil content of High Oil in 1949 was more than 1% higher than the previous high record, while the mean oil content of Low Oil was the second lowest observed. The record low mean oil content in Low Oil was 0.76% in the 1947 crop.

Analyses of each generation of High Oil and Low Oil are shown graphically in Fig. 1, together with the best-fitting straight-line trend of oil content

Table 1. Effect of 50 generations of selection for oil and protein content in Illinois chemical strains of corn.

Year	Generation	High Oil	Low Oil	High Protein	Low Protein
		% oil		% protein	
1896	0	4.70		10.92	
1901	5	6.24	3.45	13.78	9.63
1906	10	7.38	2.67	14.26	8.65
1911	15	7.52	2.06	13.79	7.90
1916	20	8.51	2.07	15.66	8.68
1921	25	9.94	1.71	16.66	9.14
1926	30	10.21	1.44	18.16	6.50
1931	35	11.80	1.23	20.14	7.12
1936	40	10.16	1.24	22.92	7.99
1941	45	13.73	1.02	17.76	5.79
1949	50	15.36	1.01	19.45	4.91

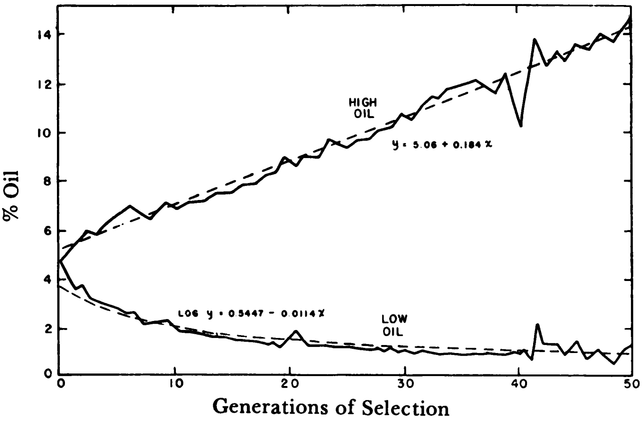

Figure 1. Effect of 50 generations of selection on oil content of corn. Actual data indicated by solid lines; fitted trend lines are shown as broken lines.

in High Oil and the best-fitting curvilinear trend of oil content in Low Oil. It is apparent that progress toward high oil content in High Oil has been made at a remarkably uniform rate throughout the entire course of the experiment. In Low Oil, on the other hand, relatively little progress toward low oil content has been made in the last 15 to 20 generations of selection. Variation from season to season has been low in both strains, with 1937 (forty-first generation) being virtually the only year in which major fluctuations from the general trends were observed.

Selection for Protein Content

The mean protein content of the foundation seed lot was 10.92%. The mean protein content of High Protein in 1949 was 19.45%, that of Low Protein was 4.91% (Table 1). The range between the means of the two strains, 14.54%, was the second greatest on record. The greatest range observed during the course of the experiment was 14.93% in 1936 (fortieth generation). The mean protein content of the 1949 crop of Low Protein was the lowest

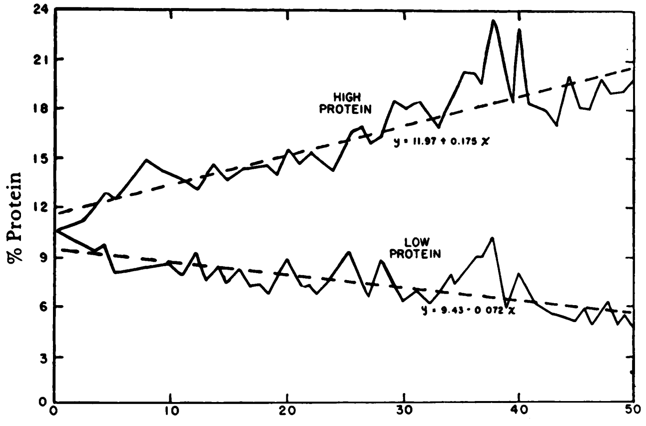

Figure 2. Effect of 50 generations of selection on protein content of corn. Actual data indicated by solid lines; fitted trend lines are shown as broken lines.

on record, while that of High Protein was the eighth highest observed during the course of the experiment. The highest mean protein content reached in High Protein was 23.79%, in the drouth year of 1934 (thirty-eighth generation).

The actual analyses and best-fitting straight-line trends of protein content in the two protein strains are shown graphically in Fig. 2. Progress toward low protein content in Low Protein was very slow during the first 25 generations of selection (6), but has been more rapid and consistent during the second 25 generations. It appears that little progress toward high protein content has been made in High Protein in the last 15 generations of the experiment.

Variations in protein content from season to season have been much more pronounced than those in oil content. This observation has been made repeatedly during the course of the experiment (4, 6). Extremely high levels of protein content in both strains during the drouth years of 1933, 1934, and 1936 (generations 37, 38, and 40, respectively) indicate the magnitude of environmental effects on protein content.

Variability Within Selected Strains

As pointed out by Winter (6), selection for oil and protein content was expected not only to shift the mean chemical content of the selected strains toward the desired type, but also to reduce variability in chemical composition within each strain. From an examination of the results of the first 28 generations of the experiment, Winter concluded that variability in oil and protein content depended on the size of the mean of the selected character. When measured by the coefficient of variation, variability was found to decrease in both high strains and to increase in both low strains. When meas-

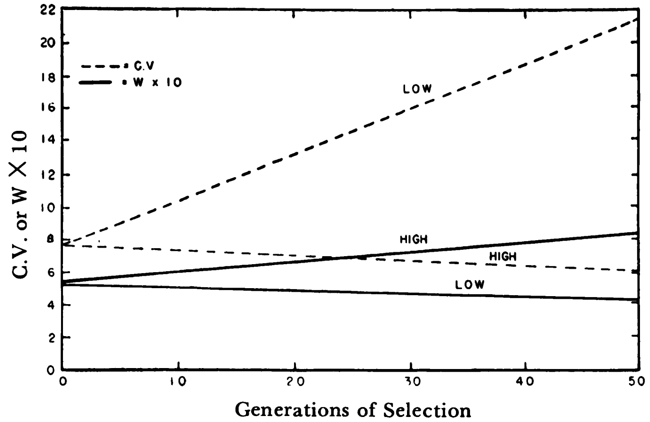

Figure 3. Straight-line trends of coefficient of variation *(C. V.)* and Weinberg constant *(W)* in Illinois High Oil and Low Oil corn. Trends of *C. V.* indicated by broken lines; those of *W* by solid lines.

ured by the standard deviation, Weinberg's formula (5), or the extramodal coefficient, variability was found to increase in both high strains and to decrease in both low strains. Winter suggested that the apparent increase in variability of the high strains probably resulted from the presence of more material for interaction with the environment.

Variability within the selected strains has been studied for the entire 50 generations of the experiment by use of the standard deviation, coefficient of variation, and Weinberg's formula. The constant *W* calculated by the latter formula is:

$$W = \frac{[s\ (\text{highest variate} - \text{lowest variate})]^{1/2}}{[(\text{highest variate} - \text{mean})(\text{mean} - \text{lowest variate})]^{1/2}}$$

where s = the standard deviation of the sample. The size of *W* tends to parallel that of the standard deviation, but it is also influenced by the skewness of the distribution. Skewness is roughly indicated by the denominator of the formula.

Figs. 3 and 4 show the best-fitting straight-line trends of the coefficient of variation and *W* for the four selected strains. In the oil strains, the only apparent major change in variability has been a very marked increase in the coefficient of variability of Low Oil. Variability, as measured by either the coefficient of variation or by *W*, has remained virtually unchanged in High Oil. Also, there has been very little change in *W* in Low Oil. In general, the trends in variability in the protein strains for the entire 50 generations have been similar to those found by Winter for the first 28 generations (6). The coefficient of variation has increased slightly in High Protein and decreased slightly in Low Protein. The size of *W* has increased considerably in High Protein and decreased in Low Protein.

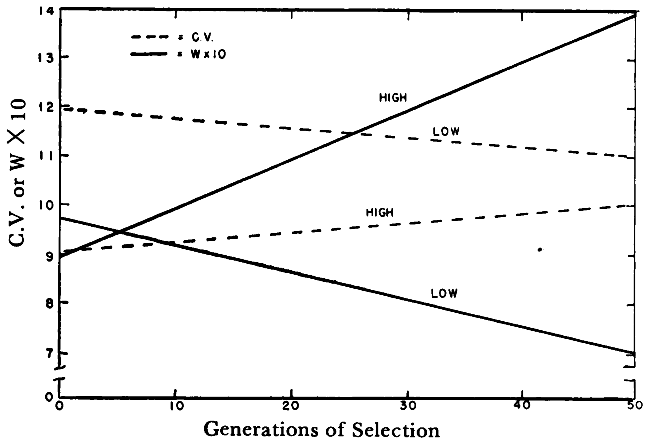

Figure 4. Straight-line trends of coefficient of variation *(C. V.)* and Weinberg constant *(W)* in Illinois High Protein and Low Protein corn. Trends of *C. V.* indicated by broken lines; those of *W* by solid lines.

Effect of Random Selection

Beginning with seed from the 1933 crop, a system of random or "nonselection" was compared with the regular selections in each of the four chemical strains for 8 years (1934 to 1941). The randomly-selected material was handled in exactly the same manner as the regular selections, except that no selection for chemical composition was made in choosing seed ears for each new generation.

Results of eight generations of random breeding are shown in Table 2. In all four strains, the nonselected groups showed some tendency to revert toward the composition of the original Burr White variety. This tendency was most pronounced in High Oil, where the nonselected group had a mean oil content for the 8-year period of 1.49% less than that of the regular selec-

Table 2. Selection compared with random or nonselection in the Illinois chemical strains of corn, 1934-1941.

Year	High Oil		Low Oil		High Protein		Low Protein	
	Selected	Non-selected	Selected	Non-selected	Selected	Non-selected	Selected	Non-selected
	% oil				% protein			
1934	11.36	10.79	1.04	1.16	23.79	22.42	10.73	11.42
1935	12.27	9.57	1.32	1.19	17.71	17.33	5.90	7.09
1936	10.14	9.36	1.24	1.19	22.92	20.34	9.61	9.30
1937	14.11	12.27	2.88	2.27	18.57	17.54	6.15	6.81
1938	13.14	12.10	1.34	2.52	17.57	18.15	5.80	6.35
1939	12.61	11.37	1.37	1.82	15.64	16.16	5.37	5.74
1940	12.57	11.04	1.36	1.77	19.92	19.25	6.02	8.31
1941	13.73	11.51	1.02	1.47	17.76	17.69	5.79	6.45
Mean	12.49	11.00	1.45	1.67	19.24	18.61	6.92	7.68

Table 3. Effect of reversing direction of selection after 48 generations of selection in Illinois chemical strains of corn.

Year	High Oil		Low Oil		High Protein		Low Protein	
	Regular selection	Reverse selection	Regular selection	Reverse selection	Regular selection	Reverse selection	Regular selection	Reverse selection
	% oil				% protein			
1947	13.45		0.76		19.24		5.11	
1948	14.25	13.45	1.04	1.10	19.14	18.20	5.50	5.53
1949	15.36	13.32	1.01	1.03	19.45	18.81	4.91	5.62

tion. Average differences between the two breeding systems in the other three strains were 0.22% in Low Oil, 0.63% in High Protein, and 0.76% in Low Protein.

Effect of Reversing Direction of Selection

To determine if genetic variability still existed within the long-time selected strains, a "reverse selection" phase of the experiment was begun in 1948. In addition to the regular seed selections made from the 1947 crop, the six ears of each strain most extreme in composition in the opposite direction to the regular selection were chosen to begin the reverse strains. For example, the six ears of Illinois High Oil lowest in oil content were chosen as the foundation seed stock for the "Low" High Oil strain. Reverse selections have since been handled in exactly the same manner as the regular strains, except that selection for chemical composition has been toward that of the original Burr White variety.

Results of the first two generations of reverse selection are shown in Table 3. As was the case in the random-breeding phase of the experiment, High Oil has been more affected by a change in the direction of selection than have the other three strains. In 1949, the reverse strain of High Oil was 2.03% lower in oil content than the regular selection. Progress has also been made by reverse selection in High Protein and Low Protein, although to a lesser degree. Two years of reverse selection have produced no apparent change in the mean oil content of Low Oil. It is intended to continue the reverse selection phase of this experiment for a number of generations.

Morphological Characters and Yield of Selected Strains

Very early in the experiment, it was recognized that selection for chemical composition was also leading to changes in the physical characteristics of the ears and kernels of the selected strains, and was also tending to reduce their yields of grain, particularly in High Protein (4). These changes have become more pronounced as selection has progressed, until at the present time each of the strains has a distinctive kernel type (Fig. 5) and ear type (Fig. 6). [Fig. 5 was also shown by Woodworth and Jugenheimer (7)]. High Oil has a relatively small ear and a small kernel with a large germ. Low Oil

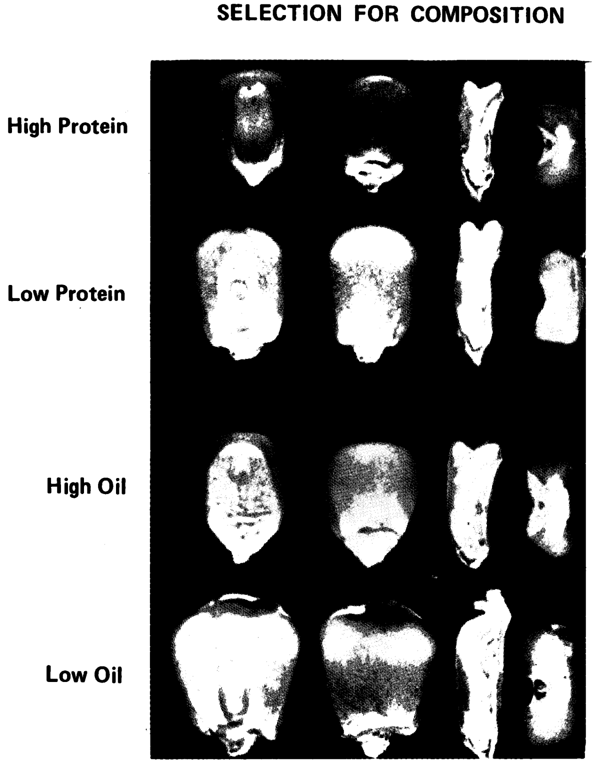

Figure 5. Kernel characteristics of Illinois chemical strains of corn. High Protein and Low Protein differ greatly in proportions of horny and soft endosperm starch; High and Low Oil differ greatly in kernel size and germ volume. Photographed by Dr. W. J. Mumm (now Director of Research, Crow Hybrid Corn Co., Milford, Ill.). Also shown as text figure by Woodworth and Jugenheimer (7).

has a large ear with low row number, and a very large, deeply dented kernel with a small germ. Ears of High Protein are small, with large cobs, and the kernels are hard, flinty, and translucent, with a medium dent. Ears of Low Protein are large, with a relatively high row number, and the kernels are long and starchy, with very little or no dent.

The four strains also differ in maturity, plant height, ear height, tillering tendency, and susceptibility to leaf firing (Table 4). High Oil is the earliest and shortest of the four strains, while Low Oil is the latest and tallest, and the two protein strains are intermediate in both maturity and height. High Protein and Low Oil both tend to tiller strongly, and both are rather susceptible to leaf firing under deficienty of available nitrogen.

Grain yields of all four strains have been reduced so that they are approximately 50% as much as those of adapted hybrids (Table 4). Smith (4) pointed out this tendency toward lower yield at the end of 10 generations of selection, but was able to show that High Protein, despite its low yield, pro-

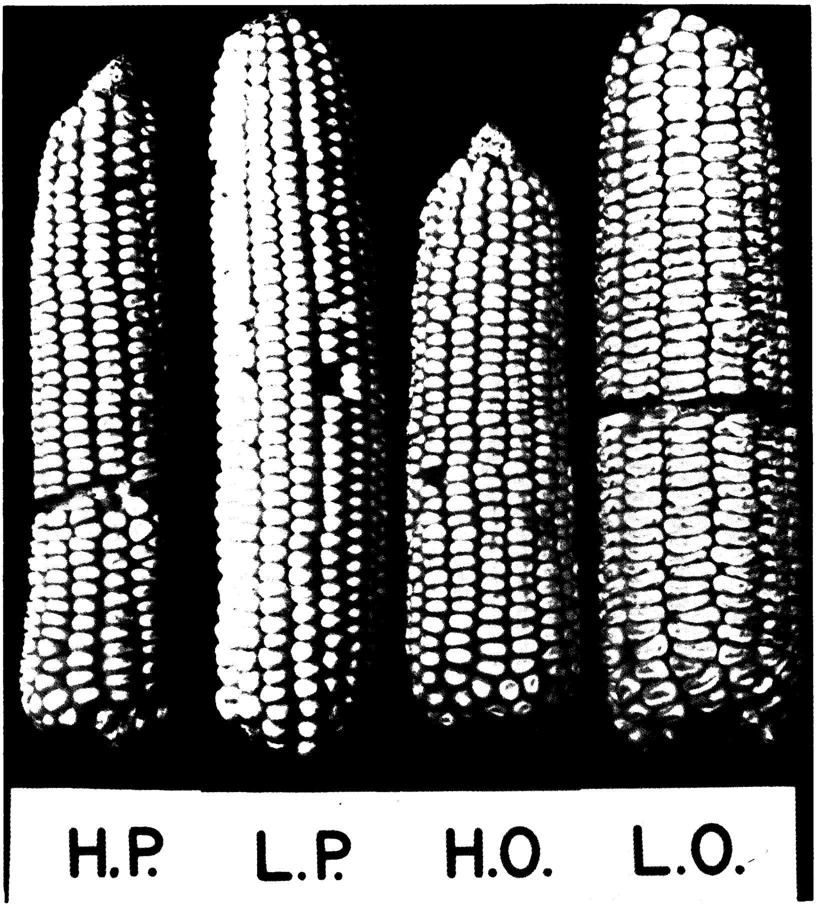

Figure 6. Ear characteristics of Illinois chemical strains of corn. *Left to right:* High Protein, Low Protein, High Oil, and Low Oil.

duced substantially more total protein per acre than the original variety. Tables 5 and 6 compare the total production of oil and protein per acre of the four chemical strains with that of an adapted commerical hybrid, a standard single cross, and three-way crosses involving inbred lines to which high oil or

Table 4. Yield and other agronomic characters of Illinois chemical strains of corn, compared with hybrid U.S. 13, Urbana, Ill., 1949.

Strain or hybrid	Yield, bu/acre	Days from planting to half-silk	Erect plants, %	Plant height, feet	Ear height, inches	Tillers/ 100 plants	Leaves fired, Aug. 24
Illinois High Oil	55	61	66	5.9	36	6	1
Illinois Low Oil	56	76	44	8.6	55	30	3
Illinois High Protein	51	72	49	6.8	45	46	5
Illinois Low Protein	56	69	75	7.5	48	7	0
U.S. 13	106	68	76	8.8	54	13	2

Table 5. Comparison of Illinois High Oil and Low Oil strains of corn with commercial hybrids and high-oil three-way crosses, for yield, oil content, and total production of oil per acre, Urbana, Ill., 1949.

Strain or hybrid	Yield, bu/acre	Oil content, %	Lb. oil/ acre
Illinois High Oil	55	12.7	330
Illinois Low Oil	56	1.0	26
U.S. 13	106	4.5	225
WF9 X Hy	103	4.1	200
Ill. 4002*	102	6.1	294
Ill. 4006†	104	5.9	290

* (WF9 X Hy) X [(H.O. X 38-11) X H.O.] S_4. † (WF9 X Hy) X [(H.O. X 187-2) X H.O.] S_4.

Table 6. Comparison of Illinois High Protein and Low Protein strains of corn with commercial hybrids and high-protein three-way crosses, for yield, protein content, and total production of protein per acre, Urbana, Ill., 1949.

Strain or hybrid	Yield, bu/acre	Protein content, %	Lb. protein/ acre
Illinois High Protein	51	17.2	415
Illinois Low Protein	56	5.4	143
U.S. 13	106	9.8	491
WF9 X Hy	103	9.5	463
Ill. 4013*	88	12.3	512
Ill. 4016†	100	10.8	512

* (WF9 X Hy) X [(HP X 38-11) X HP] S_4. † (WF9 X Hy) X [(HP X 187-2) X HP] S_4.

high protein has been transferred from Illinois High Oil or High Protein (7). From these data, it appears that Illinois High Oil, despite its greatly reduced grain yield, produced more pounds of oil per acre than commercial hybrids or the best three-way crosses involving one high oil line. Illinois High Protein, on the other hand, was exceeded in the total amount of protein produced per acre by both the high-protein three-way crosses and by both standard hybrids.

SUMMARY

Fifty generations of selection in the Illinois chemical strains of corn were completed in 1949. Selection for both high and low oil and high and low protein content was begun in the Burr White variety in 1896. Ear-to-row selection was practiced for the first 28 generations, while mass selection with intra-strain controlled cross-pollination has been employed for the last 22 generations.

The original variety had a mean oil content of 4.70% and a mean protein content of 10.92% in 1896. After 50 generations of selection, the mean oil content of Illinois High Oil was 15.36%, that of Illinois Low Oil 1.01%;

the mean protein content of Illinois High Protein was 19.45%, that of Illinois Low Protein was 4.91%. Progress in the direction of selection is apparently still being made in the High Oil and Low Protein strains, while little progress has been made in either High Protein or Low Oil in the last 15 to 20 generations of selection.

Variability, as measured by either the coefficient of variation or the Weinberg formula, has remained virtually unchanged in High Oil. The coefficient of variation of Low Oil has increased markedly, while the Weinberg constant has shown little change. High Protein has shown an increase and Low Protein a decrease in both measures of variability as a result of selection.

Eight generations of random breeding were compared with regular selection in each of the four strains in the years 1934–1941. Average differences between the two breeding systems ranged from 0.22% in Low Oil to 1.49% in High Oil, all changes in the random-bred strains being in the direction of the original Burr White analysis.

Two generations of reverse selection, begun in 1948, have indicated that High Oil, High Protein, and Low Protein may still possess considerable genetic variability. Virtually no change in the oil content of Low Oil has thus far been made by reverse selection.

All selected strains have been considerably modified in a number of morphological and agronomic characters, and they yield approximately 50% as much grain as adapted hybrids. The high strains have been used by the Illinois and other stations in an effort to transfer high oil or high protein to standard inbred lines.

EDITOR'S NOTE

Title footnote stated: "Contribution from the Plant Breeding Division, Department of Agronomy, Illinois Agricultural Experiment Station, Urbana, Ill. Published with approval of the Director, Illinois Agricultural Experiment Station. Presented at the annual meeting of the American Society of Agronomy at Cincinnati, Ohio, November 1, 1950. Received for publication March 28, 1951."

Author footnote stated: "Professor, Assistant Professor, and Professor of Plant Genetics, respectively. The authors gratefully acknowledge the assistance of various members of the Soil Fertility Division, Department of Agronomy, in making the chemical analyses of the material discussed in this paper."

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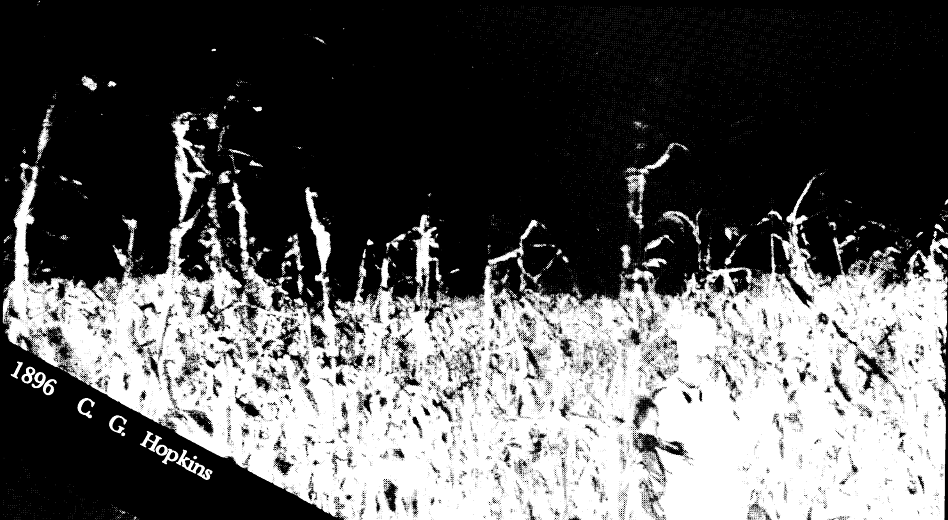
F. L. Winter 1912

Album



1896 C. G. Hopkins

1924 F. L. Winter

1924 C. M. Woodworth

Agronomy Class 1899

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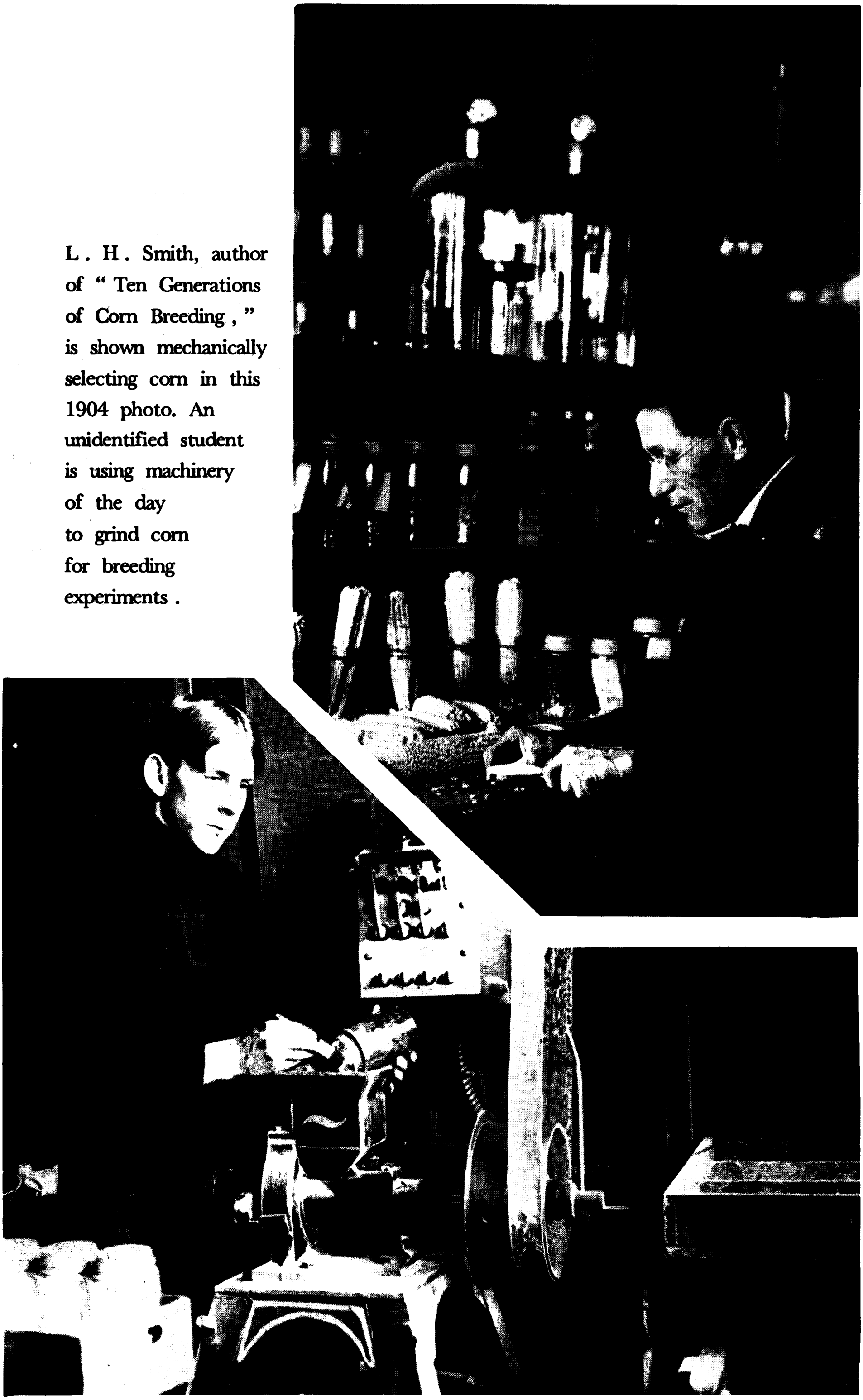

L. H. Smith, author of "Ten Generations of Corn Breeding," is shown mechanically selecting corn in this 1904 photo. An unidentified student is using machinery of the day to grind corn for breeding experiments.

6

Nitrogen Fractions of the Component Parts of the Corn Kernel as Affected by Selection and Soil Nitrogen

E. O. SCHNEIDER
E. B. EARLEY
E.E. DE TURK

INTRODUCTION

In the summer of 1944 an extensive research program was started by the Division of Soil Fertility on several phases of nitrogen metabolism of the corn plant. The program included field experiments to determine the interaction of hybrid, rate of planting, level of nitrogen fertility, and location on yield and protein content of grain; a study of the weekly absorption of soil nitrogen by the corn plant and the rate and forms of nitrogen compounds translocated into the developing grain; and a comparative study of various plant compounds and enzyme systems of corn lines differing widely in protein content of the grain.

The first object of this research program was to determine the principal factors responsible for the reported decline in the protein content of hybrid corn grain. The second object, though equally as important as the first, was to study in detail a few of the metabolic processes of the corn plant known to be associated with protein synthesis.

As a fundamental supplement to these investigations, a quantitative study was made of the various classes of protein in the germ and endosperm of six strains of corn which differed widely in total nitrogen. The results of this study are reported in this paper.

REVIEW OF LITERATURE

The only work found in the literature on the fractionation of the various proteins in different parts of the corn kernel is that of Osborne and Mendel (7). They used only one variety of corn which contained 2.33% total nitrogen. Total nitrogen and other elements have been determined on the various parts of the corn kernel by Hopkins, Smith, and East (5), Osborne and Mendel (7), and Earle, Curtis, and Hubbard (3).

SOURCE OF MATERIALS

Funk's G-80 corn was used in this study. (The Illinois chemical strains of corn used in this investigation were secured from the Division of Plant Breeding through the courtesy of Dr. C. M. Woodworth. Funk Bros. Seed Co., Bloomington, Ill., provided the other corn.) All strains except G—80, a commercial hybrid, have been selected for their respective chemical characteristics since 1896 (5). Nitrogen and oil analyses for these strains of corn and those discussed from the literature are given in Table 1, but only the nitrogen data will be considered in this paper.

The grain from Illinois High and Low Protein and Illinois High and Low Oil corn was produced in 1945 on the Agronomy south farm at Urbana, Ill. Funk's G—80 was produced the same year near Urbana, Ill., on two plots with different levels of soil nitrogen. One plot received no nitrogen fertilizer, and the corn from this plot is referred to as low-soil-nitrogen corn. The other plot received 2,400 pounds per acre of 21% ammonium sulfate applied on the surface and plowed under. Corn from this plot is referred to as high-soil-nitrogen corn. G—80 was planted at the rate of three kernels per hill checked in 40-inch rows and the other strains were drilled at the rate of one kernel per hill about every 12 inches in rows 40 inches apart.

DISSECTION OF KERNELS

A known number of air-dried kernels were dissected into tip cap, hull, germ and endosperm. Only a few kernels were completely dissected at one time so that the water used for softening them would not have time to dissolve the water-soluble nitrogen.

The kernels were placed in distilled water at room temperature for approximately 10 minutes, or until the tip cap and hull were easily removed with a knife. First the tip cap was removed, then the hull was pulled down to the dent end in strips and carefully pulled off so as to remove as little aleurone layer as possible. These strips served as a means of removing the hull adhering to the dent.

The germ and endosperm remained intact. They were separated after the hulled kernel had been in water for about 15 minutes, or until the germ became turgid and flexible. This point was determined by trial. The germ was removed most satisfactorily by starting at the tip end and slowly prying the turgid germ from the endosperm. This process was continued down along the sides of the germ until it was nearly free, then the germ was removed by starting at the tip cap end and pulling up. The portion of the germ that filled the small groove in the endosperm beneath the germ was removed fairly satisfactorily in this manner. A much cleaner separation was made if the germ remained whole throughout the entire operation. Extreme caution was used to remove portions of the endosperm or germ that clung to other parts. Color and luster combined with hardness were used for identification of parts in this separation.

The moist tip cap, hull, germ, and endosperm were dried overnight in a forced-draft oven at 40 C and 2 days allowed for the material to come to equilibrium with the atmosphere in the laboratory. The parts were then weighed and ground in a small Wiley mill equipped with a 20-mesh screen. After grinding, each part was thoroughly mixed and stored in a 6-ounce bottle.

Moisture was determined on a ground sample of each part of the kernel by drying in a vacuum oven at 76 C for 5 hours. The chemical data are reported on the moisture-free basis.

ANALYTICAL METHODS

Total Nitrogen

Ground samples of whole corn and of the parts which had been used for moisture determinations were also used for total nitrogen as determined by the official Gunning method (1).

Fractionation of the Total Nitrogen

Nitrogen was extracted from each sample with the following solvents in succession: water, 0.5 *M* sodium sulfate, 71% ethanol, and sodium hydroxide of pH 12.5. The remaining nitrogen in the sample was determined and designated insoluble nitrogen.

A ground sample of approximately 2.5 g of germ and 4 g of endosperm or whole corn was used for fractionation. The sample was placed in a 125-ml Truog pyrex centrifuge tube and kept there throughout the succession of extractions. The final sample residue was transferred into a Kjeldahl flask and oxidized for determination of the insoluble nitrogen.

The combined extracts of each solvent, which were decanted into Kjeldahl flasks, were evaporated nearly to dryness on a steam bath or on the electric heater of the Kjeldahl apparatus after receiving 5 ml of 18 *M* sulfuric acid to prevent loss of nitrogen. Total nitrogen was then determined by the Gunning method.

Water-soluble nitrogen. Approximately 60 ml of distilled water, at room temperature, was added to each of duplicate samples and the tubes stoppered and shaken on a mechanical shaker for 1 hour. The tubes were removed, and any material clinging to the stopper and sides of the tube was washed into the solution with a small amount of distilled water. The tubes were then centrifuged at 2000 rpm for 10 minutes and the supernatant liquid decanted into a Kjeldahl flask. This procedure was repeated two other times. Additional extractions did not remove significant amounts of nitrogen, as found by preliminary trials.

Salt-soluble nitrogen. About 60 ml of 0.5 *M* sodium sulfate solution, at room temperature, was added to each of the tubes containing the water-extracted samples. The salt-soluble nitrogen was removed in a manner identical to that used for the water-soluble nitrogen.

Alcohol-soluble nitrogen. About 60 ml of 71% ethanol (250 ml of distilled water and 750 ml of 95% ethanol) by volume was added to each tube and thoroughly stirred into the sample. The tubes were then transferred into a constant-temperature water bath at 55C, attached to reflux condensers, and left overnight. They were then removed, centrifuged, and the alcohol decanted into Kjeldahl flasks. The second and third extractions were made at room temperature with continuous shaking for 1 hour each, using the same amount and concentration of ethanol as in the first extraction.

Alkali-soluble nitrogen. About 60 ml of sodium hydroxide (approximately 0.2%) adjusted to pH 12.5 was added to each tube. The tubes were stoppered and shaken for 1 hour on the mechanical shaker. The centrifuging and the other two 1-hour extractions were carried out as described for the water and salt extractions.

Insoluble nitrogen. The nitrogen remaining in the sample was determined by the Gunning method and referred to as insoluble, or residual, nitrogen.

Table 1. Nitrogen and oil content of the six strains of corn used in this work and of the corn discussed from the literature.

	Original parents* (1896)	Ill. High Protein		Ill. Low Protein		Ill. High Oil		Ill. Low Oil		Funk's G-80 §		Commercial hybrids ‖	White corn ¶
		†	‡	†	‡	†	‡	†	‡	h-s-n	l-s-n		
N, %	1.75	2.06	2.72	1.48	1.06	1.81	2.08	1.60	1.86	1.71	1.46	1.65	2.33
Oil, %	4.70	5.36	5.15	4.20	3.13	7.00	13.01	2.52	1.42	4.73	4.62	4.80	—

* Original corn from which Illinois High Protein, High Oil, Low Protein, and Low Oil strains were selected.
† Hopkins, *et al.* 1903. (After 7 years of selection).
‡ Schneider, *et al.* 1945. (After 49 years of selection).
§ H-s-n is high soil nitrogen. L-s-n is low soil nitrogen.
‖ Earle, *et al.* 1946.
¶ Osborne and Mendel. 1914.

RESULTS AND DISCUSSION

The six strains of corn used in this investigation and those discussed from the literature are listed in Table 1 with their percentages of total nitrogen and oil.

The discussion of all comparative data for the Illinois High and Low Protein and Illinois High and Low Oil strains is based on the 43-year selection period (from 1903 to 1945). Comparative data for G—80 are based on the corn grown on two levels of soil nitrogen during the 1945 season.

Percentage Distribution of the Component Parts of the Corn Kernel

The data from this investigation and those from the literature, giving the distribution of the component parts of the corn kernel in terms of percentages, are presented in Table 2. For the six strains of corn investigated the endosperm averaged 80.90% of the weight of the whole kernel, the germ 11.71%, the hull 6.37%, and the tip cap 1.02%.

Forty-three years of selection for high protein resulted in an increase in the proportion of hull and germ and a decrease in tip cap and endosperm in the kernel. The increase in germ amounted to 6.37%, and the decrease in endosperm, 2.59% of the original proportions. Selecting for low protein during the same period resulted in an inverse variation, namely, an increase in the proportion of hull and endosperm and a decrease in that of the tip cap and germ. The amount of endosperm increased 0.93% while the germ decreased 12.41%.

The weight of the germ of Illinois High Protein corn was 24.40% greater than the germ of Illinois Low Protein corn in 1903, and 51.07% greater in 1945.

The result of selection for high and low oil was evidenced by an increase of 61.42% in the proportion of germ in the High Oil kernel and a decrease of 10.85% in that of the Low Oil kernel. No data are available for the other parts of the kernels for 1903, or for the original corn in 1896.

In 1903 the germ of Illinois High Oil corn made up 78.81% more of the weight of the kernel than did the germ of Illinois Low Oil corn, and in 1945 it made up 223.77% more.

Table 2. Percentage nitrogen in component parts of the corn kernel.

Strain	Parts	Percent of kernel found in parts		Percent increase	Percent nitrogen in parts		Percent increase	Percent nitrogen of whole kernel found in parts		Percent increase
		1903*	1945		1903*	1945		1903*	1945	
Illinois High Protein	Tip cap	1.62	1.06	—34.6	0.74	0.13	—82.4	0.66	0.05	—92.4
	Hull	6.09	7.94	30.4	0.61	0.67	9.8	1.84	1.96	6.5
	Germ	11.93	12.69	6.4	3.13	3.26	4.2	18.48	15.21	—17.7
	Endosperm	80.37	78.30	— 2.6	1.99	2.87	44.1	79.18	82.62	4.3
	Whole kernel	——	——	——	2.06	2.72	32.0	——	——	——
Illinois Low Protein	Tip cap	1.20	0.99	—17.5	1.18	0.13	—89.0	0.90	0.12	—86.7
	Hull	5.47	6.11	11.7	0.80	0.85	6.2	2.74	4.90	78.8
	Germ	9.59	8.40	—12.4	3.18	2.87	— 9.8	19.30	22.74	17.8
	Endosperm	83.72	84.50	0.9	1.46	0.89	—39.0	77.37	70.95	— 8.3
	Whole kernel	——	——	——	1.48	1.06	—30.4	——	——	——
Illinois High Oil	Tip cap	——	0.99	——	——	1.37	——	——	0.65	——
	Hull	——	5.34	——	——	0.86	——	——	2.21	——
	Germ	13.84	22.34	61.4	2.83	2.48	—12.4	21.64	26.64	23.1
	Endosperm	——	71.33	——	1.66	2.08	25.3	——	71.33	——
	Whole kernel	——	——	——	1.81	2.08	14.9	——	——	——
Illinois Low Oil	Tip cap	——	1.03	——	——	1.28	——	——	0.71	——
	Hull	——	5.50	——	——	0.79	——	——	2.34	——
	Germ	7.74	6.90	—10.8	3.47	3.74	7.8	16.79	13.87	—17.4
	Endosperm	——	86.57	——	1.46	1.79	22.6	——	83.31	——
	Whole kernel	——	——	——	1.60	1.86	16.2	——	——	——
Funk's G-80 (H. S. N.)	Tip cap	——	1.05	——	——	0.15	——	——	0.92	——
	Hull	——	7.21	——	——	0.83	——	——	3.50	——
	Germ	——	10.24	——	——	2.95	——	——	17.67	——
	Endosperm	——	81.50	——	——	1.66	——	——	79.12	——
	Whole kernel	——	——	——	——	1.71	——	——	——	——
Funk's G-80 (L. S. N.)	Tip cap	——	0.99	——	——	0.15	——	——	1.02	——
	Hull	——	6.10	——	——	0.63	——	——	2.63	——
	Germ	——	9.68	——	——	2.83	——	——	18.76	——
	Endosperm	——	83.23	——	——	1.38	——	——	78.67	——
	Whole kernel	——	——	——	——	1.46	——	——	——	——
White* corn	Tip cap	——	8.50	——	——	1.52	——	——	5.54	——
	Hull	——		——	——		——	——		——
	Germ	——	11.00	——	——	3.42	——	——	16.14	——
	Endosperm	——	80.50	——	——	2.28	——	——	78.77	——
	Whole kernel	——	——	——	——	2.33	——	——	——	——
Commercial hybrids*	Tip cap	——	0.80	——	——	1.45	——	——	0.70	——
	Hull	——	5.30	——	——	0.60	——	——	1.93	——
	Germ	——	11.90	——	——	3.00	——	——	21.64	——
	Endosperm	——	81.90	——	——	1.50	——	——	74.45	——
	Whole kernel	——	——	——	——	1.65	——	——	——	——

* Data taken from the literature.

Funk's G—80, under conditions of high soil nitrogen, produced a higher proportion of tip cap, hull, and germ and a lower proportion of endosperm than when grown with low soil nitrogen. The percentage increase in germ amounted to 5.78% (Table 2).

These data show that growing corn with high soil nitrogen and selecting for high protein and high oil resulted in an increase in the proportion of germ and a decrease in endosperm, whereas growing corn with low soil nitrogen and selecting for low protein and low oil gave the opposite results, namely, an increase in the proportion of endosperm and a decrease in germ. This was an unexpected modification of the germ and endosperm in protein selection since the physical changes did not correlate with the principal changes in nitrogen content.

Percent Nitrogen of the Component Parts of the Corn Kernel

The percentage of nitrogen in the component parts of the corn kernels studied in this investigation and those reported in the literature are given in Table 2.

It will be observed that for each variety of corn the germ contained the highest concentration of nitrogen with the endosperm next in order, and with the hull and tip cap having the lowest concentrations of nitrogen. The average percent nitrogen for the parts of the kernels for the six strains of corn was as follows: germ, 3.02; endosperm, 1.78; hull, 0.77; and tip cap, 0.54.

Selecting for high protein resulted in a 44.22% increase in concentration of endosperm nitrogen and a 4.15% increase in germ nitrogen. Selecting for low protein brought about a 39.04% reduction in concentration of endosperm nitrogen and 9.75% reduction in germ introgen. These data show that endosperm nitrogen is principally involved in either increasing or decreasing the concentration of grain nitrogen through selection.

Approximately the same concentration of germ nitrogen was found in both protein strains in 1903, but in 1945 Illinois High Protein had a 13.59% greater concentration of germ nitrogen than Illinois Low Protein. There was a much greater difference in the concentration of endosperm nitrogen than there was in germ nitrogen in 1903 and 1945 for these two strains of corn. In 1903 and 1945 the endosperm of Illinois High Protein contained 36.30% and 222.47% greater concentrations of nitrogen, respectively, than the endosperm of Illinois Low Protein.

Selecting for high and low oil resulted in a 12.37% reduction in germ nitrogen and a 25.30% increase in endosperm nitrogen for the High Oil strain, and a 7.78% increase in germ nitrogen and a 22.60% increase in endosperm nitrogen for the Low Oil strain. The concentration of the germ nitrogen was affected to a smaller extent than that of the endosperm nitrogen in selecting for high or low oil grain.

The concentration of nitrogen in the germ of Illinois High Oil corn was 18.44% lower in 1903 and 33.68% lower in 1945 than the germ nitrogen of Illinois Low Oil corn. Also, the concentration of nitrogen in the endosperm of Illinois High Oil corn was 13.70% higher in 1903 and 16.20% higher in 1945 than the endosperm nitrogen of Illinois Low Oil corn.

Funk's G—80 corn grown with high soil nitrogen contained a higher concentration of nitrogen in all parts of the kernel, except the tip cap, than when grown with low soil nitrogen. The increases for germ, endosperm, and hull were 4.24%, 20.29%, and 31.74%, respectively.

Distribution of the Total Nitrogen of the Corn Kernel among the Component Parts

The percentage of the total nitrogen of the whole kernel in the component parts as determined in this study and reported in the literature is given in Table 2. Results from this investigation showed that the endosperm averaged 77.67% of the nitrogen in the whole kernel, the germ 19.15%, the hull 2.92%, and the tip cap 0.58%.

Selecting for high protein during the 43-year period resulted in a 17.69% decrease in proportion of the total nitrogen of the kernel in the germ and a 4.34% increase in the endosperm. Selecting for low protein during this time brought about a 17.82% increase in proportion of the total nitrogen of the kernel in the germ and an 8.30% decrease in the endosperm.

In 1903 and 1945 the germ of Illinois High Protein corn contained 4.25% and 33.11% less of the nitrogen of the kernel, respectively, than the germ of Illinois Low Protein corn. For these same years the proportion of the total nitrogen in the endosperm of Illinois High Protein corn was 2.34% and 16.45% higher, respectively, than in the endosperm of Illinois Low Protein corn.

Selecting for high and low oil brought about an increase of 23.10% in the proportion of germ nitrogen in Illinois High Oil corn and a decrease of 17.39% in the proportion of germ nitrogen in Illinois Low Oil corn. No data are available for the proportion of the total nitrogen of the kernel contained in the endosperm of these two strains of corn.

In 1903 the proportion of the total nitrogen of the kernel in the germ of Illinois High Oil corn was 28.89% greater than that in the germ of Illinois Low Oil corn and in 1945 it was 92.07% greater.

Funk's G—80 grown on high soil nitrogen contained a greater proportion of the total nitrogen of the kernel in the endosperm and hull and a smaller proportion in the germ and tip cap than when grown on low soil nitrogen.

Nitrogen Fractions in Component Parts of the Corn Kernel

Nitrogen fractions were obtained on all parts of the corn kernel except the tip cap (Table 3). Hulls from only the high- and low-protein strains were fractionated.

It will be observed from the data in Table 3 that a higher concentration of each nitrogen fraction was found in the germ and endosperm of Illinois High Protein corn than in the corresponding parts of the Low Protein corn. The greatest difference was found between the concentrations of alcohol-soluble nitrogen in the endosperm where Illinois High Protein corn had over six times as great a concentration as the Low Protein corn.

The data for the oil strains show that the germ of Illinois High Oil corn had lower concentrations of the nitrogen fractions than the germ of the Low Oil corn. With the exception of the salt-soluble fraction, the endosperm of the High Oil strain contained a higher concentration of the nitrogen fractions than the endosperm of the Low Oil strain.

The data on Funk's G—80 grain from the high- and low-soil-nitrogen plots show that the germ from the former had a slightly higher concentration of water-soluble, salt-soluble, and insoluble nitrogen, and a slightly lower concentration of alcohol- and alkali-soluble nitrogen than from the latter. On the other hand, the endosperm of the grain from the high-soil-nitrogen plot had a higher concentration of nitrogen in all fractions except the alkali-soluble.

Table 3. Nitrogen fractions in different parts of the corn kernel expressed as percentage of the respective part and of the whole kernel.

Strain	Part of kernel	Water-soluble nitrogen		Salt-soluble nitrogen		Alcohol-soluble nitrogen		Alkali-soluble nitrogen		Insoluble nitrogen		Total nitrogen of parts		Total nitrogen of parts based on weight of whole kernel	
		Part	Whole kernel	Part	Whole kernel	Part	Whole kernel	Part	Whole kernel	Part	Whole kernel	Addition	Analysis	Addition	Analysis
Illinois High protein corn	Tip cap	—	—	—	—	—	—	—	—	—	—	—	0.132	—	0.001
	Hull	0.105	0.008	0.090	0.007	0.059	0.005	0.079	0.006	0.334	0.026	0.667	0.670	0.052	0.053
	Germ	1.722	0.218	0.509	0.065	0.105	0.013	0.230	0.029	0.869	0.110	3.435	3.260	0.435	0.414
	Endosperm	0.074	0.058	0.084	0.066	1.470	1.151	0.508	0.398	0.692	0.542	2.828	2.870	2.215	2.247
	Total		0.284		0.138		1.169		0.433		0.678			2.702	2.715
	Whole kernel		0.315		0.128		1.188		0.468		0.703			2.802	2.720
Illinois Low protein corn	Tip cap	—	—	—	—	—	—	—	—	—	—	—	0.134	—	0.001
	Hull	0.140	0.009	0.137	0.008	0.055	0.003	0.104	0.006	0.412	0.025	0.849	0.850	0.051	0.052
	Germ	1.500	0.126	0.228	0.019	0.103	0.009	0.122	0.010	0.832	0.070	2.795	2.870	0.234	0.241
	Endosperm	0.054	0.046	0.050	0.042	0.249	0.210	0.360	0.304	0.199	0.168	0.912	0.892	0.770	0.754
	Total		0.181		0.069		0.222		0.320		0.263			1.055	1.048
	Whole kernel		0.193		0.087		0.169		0.332		0.313			1.094	1.060
Illinois High oil corn	Tip cap	—	—	—	—	—	—	—	—	—	—	—	1.370	—	0.014
	Hull	—	—	—	—	—	—	—	—	—	—	—	0.864	—	0.045
	Germ	1.618	0.361	0.248	0.055	0.088	0.020	0.162	0.036	0.444	0.099	2.560	2.480	0.571	0.554
	Endosperm	0.092	0.066	0.077	0.055	1.036	0.739	0.403	0.287	0.475	0.339	2.083	2.080	1.486	1.484
	Total		0.427		0.110		0.759		0.323		0.438			2.057	2.097
	Whole kernel		0.531		0.131		0.773		0.346		0.314			2.095	2.080
Illinois Low oil corn	Tip cap	—	—	—	—	—	—	—	—	—	—	—	1.280	—	0.013
	Hull	—	—	—	—	—	—	—	—	—	—	—	0.798	—	0.044
	Germ	1.628	0.112	0.498	0.034	0.166	0.011	0.318	0.022	1.169	0.081	3.779	3.740	0.260	0.258
	Endosperm	0.075	0.065	0.077	0.067	0.734	0.635	0.518	0.448	0.410	0.355	1.814	1.790	1.570	1.550
	Total		0.177		0.101		0.646		0.470		0.436			1.830	1.865
	Whole kernel		0.176		0.138		0.699		0.487		0.370			1.840	1.860
Funk's G-80 High soil nitrogen	Tip cap	—	—	—	—	—	—	—	—	—	—	—	0.148	—	0.002
	Hull	—	—	—	—	—	—	—	—	—	—	—	0.831	—	0.060
	Germ	1.606	0.164	0.236	0.024	0.125	0.013	0.152	0.016	0.726	0.074	2.845	2.950	0.291	0.302
	Endosperm	0.074	0.060	0.062	0.051	0.680	0.554	0.490	0.399	0.319	0.260	1.625	1.660	1.324	1.353
	Total		0.224		0.075		0.567		0.415		0.334			1.615	1.717
	Whole kernel		0.291		0.096		0.508		0.328		0.410			1.633	1.710
Funk's G-80 Low soil nitrogen	Tip cap	—	—	—	—	—	—	—	—	—	—	—	0.147	—	0.001
	Hull	—	—	—	—	—	—	—	—	—	—	—	0.631	—	0.038
	Germ	1.415	0.137	0.234	0.023	0.128	0.012	0.185	0.018	0.674	0.065	2.636	2.830	0.255	0.274
	Endosperm	0.069	0.057	0.056	0.046	0.442	0.365	0.520	0.428	0.183	0.150	1.270	1.380	1.046	1.135
	Total		0.194		0.069		0.377		0.446		0.215			1.301	1.448
	Whole kernel		0.231		0.096		0.364		0.442		0.250			1.383	1.460

Nitrogen of the corn germ was predominantly water soluble (Table 4). It averaged 53.0% of the total germ nitrogen for the varieties studied. The insoluble nitrogen was next highest, averaging 25.5% of the total. In each germ sample the alcohol-soluble nitrogen was the lowest of the various nitrogen fractions and constituted an average of 3.9% of the total germ nitrogen. The germ nitrogen of the six strains of corn had the following average solubility distribution: 53.0% water soluble, 10.5% salt soluble, 3.9% alcohol soluble, 6.3% alkali soluble, and 25.5% insoluble.

Corn selected for high and low protein and corn grown with high soil nitrogen varied very little from the average in the proportion of the nitrogen fractions in the germ. Selecting corn for high and low oil brought about a slight difference from the average in the proportion of salt-soluble, alcohol-soluble, and alkali-soluble nitrogen and a considerable difference in the proportion of water-soluble and insoluble nitrogen in the germ. Slightly over 65% of the total nitrogen of the High Oil germ was water soluble as compared to 43.5% for the Low Oil germ.

Corn endosperm nitrogen was predominantly alcohol soluble, averaging 40.4% of the total nitrogen of the part (Table 4). Next was the alkali-soluble which averaged 28.9% of the endosperm nitrogen. This was closely followed by the insoluble fraction with an average of 20.8%. Water- and salt-soluble nitrogen made up 4.4 and 4.0% of the endosperm nitrogen, respectively.

It is of interest to note from the data in Table 4 that as the total nitrogen of the grain was increased by selection or by addition of nitrogen to the soil, the proportion of alcohol-soluble nitrogen in the endosperm was increased and the alkali-soluble nitrogen was decreased. The proportions of the other nitrogen fractions were not altered appreciably by these two ways of increasing grain nitrogen.

Table 4. Nitrogen fractions in different parts of the corn kernel expressed as percentage of the total nitrogen in the part.

Part of kernel	Water-soluble	Salt-soluble	Alcohol-soluble	Alkali-soluble	Insoluble	Sum of fractions
	Illinois High Protein Corn (2.72% Nitrogen)					
Hull	15.7	13.4	8.8	11.8	49.8	99.5
Germ	52.8	15.6	3.2	7.0	26.6	105.2
Endosperm	2.6	2.9	51.2	17.7	24.1	98.5
	Illinois Low Protein Corn (1.06% Nitrogen)					
Hull	16.5	16.1	6.5	12.2	48.5	99.8
Germ	52.3	7.9	3.6	4.2	29.0	97.0
Endosperm	6.0	5.6	27.9	40.4	22.3	102.2
	Illinois High Oil Corn (2.08% Nitrogen)					
Germ	65.2	10.0	3.5	6.5	17.9	103.1
Endosperm	4.4	3.7	49.8	19.4	22.8	100.1
	Illinois Low Oil Corn (1.86% Nitrogen)					
Germ	43.5	13.3	4.4	8.5	31.2	100.9
Endosperm	4.2	4.3	40.8	28.9	22.9	101.1
	Funk's G-80 High Soil Nitrogen Corn (1.71% Nitrogen)					
Germ	54.4	8.0	4.2	5.2	24.6	96.4
Endosperm	4.4	3.7	41.0	29.5	19.2	97.8
	Funk's G-80 Low Soil Nitrogen Corn (1.46% Nitrogen)					
Germ	50.0	8.3	4.5	6.5	23.8	93.1
Endosperm	5.0	4.0	32.0	37.7	13.3	92.0
	Average					
Hull	16.1	14.8	7.6	12.0	49.2	99.7
Germ	53.0	10.5	3.9	6.3	25.5	99.2
Endosperm	4.4	4.0	40.4	28.9	20.8	98.5

Table 5. Percentage of the total nitrogen of each solubility fraction contained in the different parts of the corn kernel.

Part of kernel	Water-soluble	Salt-soluble	Alcohol-soluble	Alkali-soluble	Insoluble
Illinois High Protein Corn (2.72% Nitrogen)					
Hull	2.54	5.47	0.42	1.28	3.70
Germ	69.21	50.78	1.09	6.20	15.65
Endosperm	18.41	51.56	96.97	85.04	77.10
Total	90.16	107.81	98.48	92.52	96.45
Illinois Low Protein Corn (1.06% Nitrogen)					
Hull	4.66	9.20	1.78	1.81	7.99
Germ	65.28	21.84	5.32	3.01	22.36
Endosperm	23.83	48.28	124.26*	91.57	53.67
Total	93.77	79.32	131.36	96.39	84.02
Illinois High Oil Corn (2.08% Nitrogen)					
Germ	67.98	41.98	2.59	10.40	31.53
Endosperm	12.43	41.98	95.60	82.95	107.96*
Total	80.41	83.96	98.19	93.35	139.49
Illinois Low Oil Corn (1.86% Nitrogen)					
Germ	63.64	24.64	1.57	4.52	21.89
Endosperm	39.93	48.55	90.84	91.99	95.94*
Total	100.57	73.19	92.41	96.51	117.83
Funk's G-80 High Soil Nitrogen Corn (1.71% Nitrogen)					
Germ	56.36	25.00	2.56	4.88	18.05
Endosperm	20.62	53.12	109.06*	121.65*	63.41
Total	76.98	78.12	111.62	126.53	81.46
Funk's G-80 Low Soil Nitrogen Corn (1.46% Nitrogen)					
Germ	59.31	23.96	3.30	4.72	26.00
Endosperm	24.68	47.92	100.27*	96.83	60.00
Total	83.99	71.88	103.57	101.55	86.00
Average					
Hull	3.60	7.34	1.10	1.54	5.84
Germ	63.63	31.37	2.74	5.62	22.58
Endosperm	22.82	48.57	94.47	89.68	63.54
Total	90.05	87.28	98.31	96.84	91.96

* Slight error in analysis; not included in average.

These data verify the correlation between the total nitrogen of the grain and the alcohol-soluble nitrogen of the endosperm as reported by Hansen *et al.* (4).

Further study of the nitrogen fractionation data (Table 5) shows that the germ contained approximately 63% of the water-soluble nitrogen of the whole kernel, 31% of the salt-soluble, 3% of the alcohol-soluble, 5% of the alkali-soluble, and 22% of the insoluble nitrogen. The endosperm contained about 23% of the water-soluble nitrogen of the whole kernel, 48% of the salt-soluble, 94% of the alcohol-soluble, 90% of the alkali-soluble, and 63% of the insoluble nitrogen.

Nitrogen Fractions of the Whole Kernel

The average total nitrogen of the whole grain (Table 6) of the six strains of corn was divided into the various solubility fractions as follows: 16.3% water soluble, 6.5% salt soluble, 31.5% alcohol soluble, 23.5% alkali soluble, and 21.3% insoluble.

The data show that in selecting for high protein corn there was a larger proportional increase in the alcohol-soluble nitrogen than in any of the other nitrogen fractions. It is believed that fertilizing for high protein corn also gives a larger proportional increase in the alcohol-soluble nitrogen than in any of the other fractions, since the data do not clearly indicate that such is not the case. Selecting for high oil, which also resulted in a slight increase in

Table 6. Nitrogen fractions in different parts of the corn kernel expressed as percentages of the total nitrogen in the whole kernel.

Part of kernel	Water-soluble	Salt-soluble	Alcohol-soluble	Alkali-soluble	Insoluble	Sum of fractions
Illinois High Protein Corn (2.72% Nitrogen)						
Hull	0.3	0.3	0.2	0.2	1.0	2.0
Germ	8.0	2.4	0.5	1.1	4.0	16.0
Endosperm	2.1	2.4	42.3	14.6	19.9	81.3
Total	10.4	5.1	43.0	15.9	24.9	99.3
Whole corn	11.6	4.7	43.7	17.2	25.8	103.0
Illinois Low Protein Corn (1.06% Nitrogen)						
Hull	0.8	0.8	0.3	0.6	2.4	4.9
Germ	11.9	1.8	0.8	0.9	6.6	22.0
Endosperm	4.3	4.0	19.8	28.7	15.8	72.6
Total	17.0	6.6	20.9	30.1	24.8	99.5
Whole corn	18.2	8.2	15.9	31.3	29.5	103.1
Illinois High Oil Corn (2.08% Nitrogen)						
Germ	17.4	2.6	1.0	1.7	4.8	27.5
Endosperm	3.2	2.6	35.5	13.8	16.3	71.4
Total	20.6	5.2	36.5	15.5	21.1	98.9
Whole corn	25.5	6.3	37.2	16.6	15.1	100.7
Illinois Low Oil Corn (1.86% Nitrogen)						
Germ	6.0	1.8	0.6	1.2	4.4	14.0
Endosperm	3.5	3.6	34.1	24.1	19.1	84.4
Total	9.5	5.4	34.7	25.3	23.5	98.4
Whole corn	9.5	7.4	37.6	26.2	19.9	100.6
Funk's G-80 High Soil Nitrogen Corn (1.71% Nitrogen)						
Germ	9.6	1.4	0.8	0.9	4.3	17.0
Endosperm	3.5	3.0	32.4	23.3	15.2	77.4
Total	13.1	4.4	33.2	24.2	19.5	94.4
Whole corn	17.0	5.6	29.7	19.2	24.0	95.5
Funk's G-80 Low Soil Nitrogen Corn (1.46% Nitrogen)						
Germ	9.4	1.6	0.8	1.2	4.4	17.4
Endosperm	3.9	3.2	25.0	29.3	10.3	71.7
Total	13.3	4.8	25.8	30.5	14.7	89.1
Whole corn	15.8	6.6	25.0	30.3	17.1	94.8
Average of Six Lines						
Whole corn	16.3	6.5	31.5	23.5	21.3	99.6

protein content, did not modify the proportion of alcohol-soluble nitrogen from that of the Low Oil corn. It did, however, increase the proportion of water-soluble nitrogen in the whole kernel and decrease the proportion of alkali-soluble nitrogen. The surprising thing was that the alcohol-soluble nitrogen of Illinois High Oil and Illinois Low Oil corn made up essentially the same proportion of the total nitrogen of the whole kernel.

Analysis of the Distribution of the Nitrogen Difference Between the Illinois High and Illinois Low Protein Corn

Data in Table 1 show that the Low Protein corn contained 1.06% nitrogen and the High Protein corn contained 2.72% nitrogen. These values represent a difference of 1.66% nitrogen in favor of the High Protein corn. The data in Table 7 show the division of the nitrogen increase (1.66%) between germ and endosperm and the distribution of the nitrogen in each part on the basis of its solubility in the various dispersing agents.

It may be observed from these data that of the difference of 1.66% in total nitrogen considered as an increase for the High Protein corn, 10.4% of the increase occurred in the germ and 89.9% in the endosperm, with no increase going to the other parts of the kernel. Of the 10.4% increase in germ nitrogen, 53.2% of this amount was water soluble, 26.6% salt soluble, 2.3% alcohol soluble, 11.0% alkali soluble, and 23.1% insoluble. Of the 89.9% in-

Table 7. Distribution of nitrogen increase in Illinois High Protein corn over Illinois Low Protein corn among the different classes of proteins and also between the germ and endosperm.

Classes of protein	Section 1					Section 2					Section 3*			
	Germ					Endosperm					Whole kernel†			
	I L-P	II H-P	III Inc.	IV ¶	V ¶	VI L-P	VII H-P	VIII Inc.	IX ¶	X ¶	XI L-P	XII H-P	XIII Inc.	XIV ¶
	gms‡	gms‡	gms‡	%	%	gms§	gms§	gms§	%	%	gms‖	gms‖	gms‖	%
Water-soluble	0.126	0.218	0.092	53.2	5.5	0.046	0.058	0.012	0.8	0.7	0.193	0.315	0.122	7.3
Salt-soluble	0.019	0.065	0.046	26.6	2.8	0.042	0.066	0.024	1.6	1.4	0.087	0.128	0.041	2.5
Alcohol-soluble	0.009	0.013	0.004	2.3	0.2	0.201	1.151	0.941	63.0	56.7	0.169	1.188	1.019	61.4
Alkali-soluble	0.010	0.029	0.019	11.0	1.1	0.304	0.398	0.094	6.3	5.7	0.332	0.468	0.136	8.2
Insoluble	0.070	0.110	0.040	23.1	2.4	0.168	0.542	0.374	25.0	22.5	0.313	0.703	0.390	23.5
By addition	0.234	0.435	0.201	—	—	0.770	2.215	1.445	—	—	1.100	2.810	1.710	—
By analysis	0.241	0.414	0.173	—	—	0.754	2.247	1.493	—	—	1.060	2.720	1.660	—
Increase in part as per-cent of whole kernel	—	—	—	—	10.4	—	—	—	—	89.9	—	—	—	—

* The data in section 3 are not derived from section 1 and 2.

† Whole kernel including tip cap and hull. The combined value for germ and endosperm does not meet the actual totals for whole kernel because the small values for the tip cap and hull were omitted and because of the accumulated analytical errors.

‡ Grams of germ nitrogen in 100 grams of whole kernels.

§ Grams of endosperm nitrogen in 100 grams of whole kernels.

¶ Grams of nitrogen in 100 grams of whole kernels.

‖ See following for explanation.

IV. Increase of high protein over low protein in the respective classes of protein nitrogen in the germ expressed as percentage of the total germ increase found by analysis. The difference by analysis is used as the basis for calculation rather than the sum of the differences because the former is subject to fewer experimental errors.

V. Increases for high protein over low protein, as in column IV, except that the increases are computed as percentage of the overall difference in total protein nitrogen for the whole kernel instead of the germ alone, namely, 1.66%.

IX. Same as column IV except that it applies to endosperm.

X. Same as column V note except that it applies to the endosperm nitrogen.

XIV. Increase of high protein over low protein in the respective classes of protein nitrogen in the whole grain expressed as percentage of the total nitrogen increase of high protein over low protein by analysis (1.66). Difference by analysis is subject to fewer experimental errors than the sum of fractional differences and is, therefore, used as the basis for computation.

crease in endosperm nitrogen, 0.8% was water soluble, 1.6% salt soluble, 63.0% alcohol soluble, 6.3% alkali soluble, and 25.0% insoluble.

Comparison of these data with those in Table 5 shows that the increased nitrogen of the germ of Illinois High Protein corn gave essentially the same solubility pattern as did the total nitrogen of the germ. The increased nitrogen of the endosperm of the High Protein corn gave a solubility pattern which differed from that given by the total nitrogen of the endosperm. The increased nitrogen in the germ and endosperm of Funk's G—80 grain on high nitrogen soil gave the same general solubility patterns as did the increased nitrogen in the germ and endosperm of Illinois High Protein corn.

These data indicate that when the total nitrogen of corn grain is increased by selection or by nitrogen fertilization of the soil, the solubility pattern of the germ nitrogen is not appreciably modified, whereas the solubility pattern of the endosperm nitrogen is appreciably modified.

The higher proportion of alcohol-soluble nitrogen (zein) in Illinois High Protein and Funk's G—80 on high soil nitrogen, as compared to Illinois Low Protein and Funk's G—80 on low soil nitrogen, indicates that the biological value of protein of higher protein corn is less than that of protein of lower protein corn. However, recent experiments have shown that high protein corn is of greater economic value to the feeder than low protein corn (2).

Nutritional Value of the Proteins of Illinois High and Low Oil Corn Strains

The question frequently arises concerning the nutritional value of grain protein of high and low oil corn. It has been suggested that since the large germ of high oil corn contains a larger proportion of the total grain nitrogen than the small germ of low oil corn, high oil corn should be a superior protein feed (Table 2). Its protein should have a higher biological value than protein of low oil corn.

The basis for this line of reasoning is that corn germ is a high-quality protein feed (6) and corn endosperm a low-quality protein feed; consequently, an increase in size of germ at the expense of endosperm will improve the nutritional value of the protein of the whole kernel. This reasoning is based on the assumption that the increase in the proportion of germ nitrogen in high oil corn is largely at the expense of the low-quality protein of the endosperm; namely, zein.

However, the nitrogen fractionation data for the Illinois High and Low Oil corn (Table 6) do not substantiate this line of reasoning, for the increase in the proportion of germ nitrogen in the High Oil corn was at the expense of the alkali-soluble nitrogen of the endosperm and not at the expense of zein. Since the alkali-soluble protein is considered high quality (7), an increase in the proportion of germ nitrogen at the expense of alkali-soluble endosperm nitrogen may not appreciably raise the biological value of the corn protein as a whole. It will be observed (Table 6) that the alcohol-soluble nitrogen, or zein, constituted about 37% of the grain nitrogen in both the High and Low Oil strains. On this basis these two strains of corn might be expected to have about equal value as a protein feed. On the other hand, if the increase in the proportion of germ nitrogen of the High Oil corn were brought about at the expense of the alcohol-soluble rather than the alkali-soluble nitrogen of the endosperm, no one would question its superior protein feeding value over the Low Oil corn.

Work is now in progress to learn more about the nitrogen fractionation pattern of several strains of corn which have approximately the same percentage of total nitrogen, but which differ widely in content of oil.

SUMMARY

The purpose of this investigation was to determine the effect of selection and nitrogen fertilization of the soil on the weight of the component parts of the corn kernel, the percent of total nitrogen in the kernel and its parts, and the percent of nitrogen in the various solubility fractions of the whole kernel and its parts.

Kernels from six different strains of corn, Illinois High Protein, Illinois Low Protein, Illinois High Oil, Illinois Low Oil, Funk's G—80 from high soil nitrogen plots, and Funks G—80 from low soil nitrogen plots were studied.

Kernels were dissected into four parts, tip cap, hull, germ and endosperm. The component parts of all the kernels, except those from the High and Low Oil lines where the germ and endosperm had undergone remarkable changes, showed only minor differences. Percent nitrogen of the parts was in the decreasing order of germ, endosperm, hull, and tip cap.

Selecting for high protein from 1903 to 1945 resulted in an increase of 44.22% in the concentration of endosperm nitrogen and an increase of

4.15% in germ nitrogen. Selecting for low protein during the same period brought about a 39.04 and a 9.75% reduction in the concentration of endosperm nitrogen and germ nitrogen, respectively. The endosperm is principally involved in increasing or decreasing the nitrogen of the corn kernel by selection or by nitrogen fertilization of the soil.

Nitrogen of the whole kernel and of the germ and endosperm was separated into five solubility fractions: namely, water, salt, alcohol, alkali, and insoluble. Germ nitrogen was predominantly water soluble and endosperm nitrogen predominantly alcohol soluble.

All nitrogen fractions of the whole kernel usually increased when the total nitrogen of the kernel was increased by breeding or by nitrogen fertilization of the soil; however, the alcohol-soluble nitrogen (zein) increased at the fastest rate. Since zein is a low-quality protein these data indicate that the protein of high protein corn has a lower biological value than the protein of low protein corn.

An analysis of the distribution of the nitrogen increase of 1.66% for Illinois High Protein over Illinois Low Protein corn showed that 10.4% of the increase occurred in the germ and 89.9% in the endosperm.

The germ of Illinois High Oil and Illinois Low Oil corn contained 26.64 and 13.87%, respectively, of the total nitrogen of the kernel. From this it appears reasonable to conclude that protein of high oil corn has a higher biological value than that of low oil corn. However, since the alcohol-soluble nitrogen (zein) of each of these strains constituted about the same percentage of the total nitrogen of the kernel, it is suggested that there may be little or no difference in the biological value of their proteins.

EDITOR'S NOTE

The title footnote stated: "Contribution from the Department of Agronomy, Agricultural Experiment Station, University of Illinois, Urbana, Ill. Published with the approval of the Director. The major part of this work was included in a thesis submitted by the senior author in partial fulfillment of the requirements for the Master of Science degree in the Graduate College. This investigation was made possible by a cooperative gift fund from Funk Bros. Seed Co. of Bloomington, Ill.; Mr. George M. Moffett, Chairman of the Board of Corn Products Refining Co.; and the Corn Industries Research Foundation. Paper received for publication May 12, 1951."

The author footnote stated: "Graduate student, Associate Professor, and Professor of Soil Fertility, Department of Agronomy, University of Illinois, respectively."

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Results of Long-Term Selection for Chemical Composition in Maize and Their Significance in Evaluating Breeding Systems

E. R. LENG

A. INTRODUCTION

The primary objective of plant or animal breeding is the production of desired phenotypic changes in the selected material. These changes must be under hereditary control if they are to be of value. In some cases – unfornately few in proportion – the hereditary situation is simple, and the desired changes can be made quickly and stabilized readily in the new population.

Much more frequently, the hereditary control of important economic characters is complex, and its manipulation in a breeding program is difficult. It is therefore of great importance to the breeder to make use of all available genetic information and experimental evidence which may help him to make easier, greater, or more rapid progress in his breeding work. The relatively slow rate of reproduction and high cost of handling substantial populations in most domestic animals or cultivated plants makes the conduct of deliberate comparisons expensive and slow. Much useful information has been derived from long-term selection experiments with laboratory organisms, such as those with *Drosophila*, reported by Robertson (1955), Bell et al. (1955), Clayton and Robertson (1957), and others, and with mice by MacArthur (1949) and Falconer (1953, 1955). Research in breeding theory has also been pursued energetically by many workers specializing in statistical genetics. Much of the breeding theory developed by laboratory or statistical research has not been adequately evaluated under practical conditions. This is particularly true in cultivated plants, where very little critical evidence is available for testing the fundamental premises on which breeding theory should be based.

The long-term selection experiment with chemical composition in maize conducted for 61 generations at the Illinois Agricultural Experiment Station, affords unique material for evaluating breeding theory by comparison with actual observations. In addition to the obvious advantages resulting from the accumulation of data over such a long period, the following favorable aspects of this experiment should be stressed:

1. Selection was practiced for economic characters in an economically-important plant species.
2. The characters studied could be sampled, determined, and recorded easily and accurately.
3. Selection was conducted in two chemical constituents, each of which was selected for higher and lower levels by the same breeding system and with the same selection intensity.
4. Only four different persons have supervised the work from 1896 until the present time; this has insured continuity in the experimental procedure.

The purpose of this paper is to present the principal results achieved in the long-term selection experiment, and to discuss these results in their relation to some of the basic concepts of breeding theory.

B. MATERIAL AND BREEDING SYSTEMS

Selection was begun in 1896 in a population based on a well-adapted local open-pollinated variety of maize. Four selected sub-populations ("strains") were established. These have been maintained as closed breeding groups continually during the entire course of the experiment. With the exception of the years 1942, 1943, and 1944, a generation of selection has been conducted each year since 1896. Thus, the 61st generation of selection in the following four "regular" strains was completed in 1960.

"Illinois High Protein"
"Illinois Low Protein"
"Illinois High Oil"
"Illinois Low Oil"

The original breeding method, as described by Hopkins (1899) and Smith (1908) was modified several times during the course of the experiment, but the basic breeding principle — that of selecting desirable individuals and intercrossing their progeny — has remained unchanged. Weaver and Thompson (1957) have pointed out that the breeding systems employed incorporate the essential principles of "recurrent selection for a phenotypic character" as defined by Johnson (1952).

I. The Original Breeding System

The original objective of the experiment was to determine if the oil and protein content of maize could be altered by selection. Hopkins (1898) described in detail the chemical composition of the source material. A random sample of 163 open-pollinated ears was drawn from the local maize variety "Burr White," and each ear was analyzed for protein and oil content. The four selected strains were then established by selecting seed from the ears most extreme in the desired direction of chemical composition. The "High Protein" and "High Oil" strains were based on the 24 ears highest in protein or oil content, respectively. The "Low Protein" and "Low Oil" strains were based on 12 each.

Each selected strain was maintained by "ear-to-row" selection in an isolated plot. During the first 9 generations of selection, the number of ears analyzed in each strain was somewhat variable. Approximately 5 ears per row, or 120 ears per strain, were analyzed each generation. Seed taken from the 24 ears, in each strain, most extreme in the desired direction of composition was planted in the respective isolation plot, each ear in a separate row. Pedigree records were maintained to show the maternal ancestry of each selected ear.

II. Modified Ear-to-Row Selection

Declining vigor and yield in each of the selected strains called attention of the breeders to the relatively high level of inbreeding which resulted from the breeding system being employed. Since it was desired that the selected strains have satisfactory yielding ability, as well as modified chemical composition, the breeding system was altered after 9 generations of selection. From the 10th to the 25th generation of selection, alternate rows in each breeding plot were detasseled, and ears for analysis were harvested only from the detasseled rows. Moreover, selection for yielding ability was begun, and ears for analysis were saved only from the 6 highest yielding detasseled rows in each strain. Thus, only 12 of the seed ears in any given generation could provide male parents for the next generation, and only 6 (different) ears could provide the female parents.

For chemical analysis, between the 10th and 25th generations, 20 ears were harvested from each of the 6 maternal parent rows. All of these ears were then analyzed, and 24 (20%) of the analyzed ears were selected as seed for the next generation.

In 1921, the system was again altered. According to Winter (1929), this was also an attempt to reduce the amount of inbreeding. Yielding ability was disregarded, and two seed ears were chosen from each of the 12 detasseled rows.

III. Intra-Strain Reciprocal Crossing

A fundamental change in the breeding system was made in 1925, after 28 generations of ear-to-row breeding had been conducted. Partly, the changed system was made necessary by the difficulty of securing adequate isolation for the four breeding plots. In part, also, it appears that a change in the supervisory personnel[1] introduced major alterations in the evaluation of basic concepts in the study. The production of commercially-feasible varieties by the breeding methods being employed was considered impractical. However, the selection experiment was continued because of its theoretical interest and because the "high" strains were considered to be potentially valuable as parental material in future breeding programs.

Ear-to-row selection was discontinued, and a system of intra-strain reciprocal crossing between sub-strains was introduced. Natural pollination in isolated plots was also discontinued, being replaced by controlled hand-pollination. The number of ears analyzed per generation in each strain was reduced to 60, but the selection intensity was maintained at the same level (1 in 5), since 12 seed ears were selected each year to produce the next generation.

Seed ears were divided into two "lots" of 6 ears each. Seed from each lot was bulked and planted in sufficient quantity to give about 100 plants after thinning. In each

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[1] The late Professor Dr. C. M. Woodworth assumed direction of the experiment in 1921. Dr. L. H. Smith directed the project from 1900 until 1921.

strain, pollen was collected from 15 to 20 plants of "Lot I," bulked, and used to pollinate 50 to 60 ear-shoots of "Lot II." Similarly, bulked pollen of "Lot II" was used to pollinate ears of "Lot I." At harvest, 30 well-filled ears were chosen from each lot of each strain for chemical analysis. The 12 highest (or lowest) ears were then chosen for seed, but identity of the two lots was maintained to a considerable extent. This breeding procedure has been continued to the present time[2].

IV. Random Mating (Non-Selection)

Beginning with seed from the 1933 crop (37th generation), a system of random mating or "non-selection" was compared with the regular breeding system for 8 generations (1934 to 1941). The random-mated populations were of the same size and handled in the same manner as the regular selections, except that no selection for chemical composition was made.

V. Reverse Selection

Using seed from the 1947 crop (48th generation of selection), the author began selection reversal in all four strains in 1948. This phase of the experiment was begun as a study of the presence of genetic variability in the long-selected strains. From the source material, the 6 ears most extreme in chemical composition in the opposite direction to the regular selection were chosen; for example, the 6 ears of "Illinois High Oil" lowest in oil content were chosen as the foundation seed stock for the "Reverse High Oil" strain. These were divided into two lots of 3 ears each; planting, pollination, harvesting, and analytical techniques were the same as those used for the regular selections.

In subsequent generations of reverse selection, the number of ears analyzed and the number selected for seed were the same as in the regular strains. The only difference was that selection was in the direction opposite to that practiced in the regular strains – that is, it was toward the chemical composition of the original Burr White population.

The 13th generation of reverse selection was completed in 1960.

VI. Switchback Selection

After 7 generations of reverse selection, the Reverse High Oil strain was again subdivided. Reverse selection, in the manner described above, was continued; also, selection was again turned toward higher oil content. To begin the "switchback High Oil" strain, the 12 ears of Reverse High Oil with highest oil content were chosen. Population sizes and methods employed were identical with those used in "regular" and "reverse" selection. The 6th generation of "switchback" selection was completed in 1960.

C. METHODS AND CHEMICAL ANALYSIS

Sampling and analytical methods during early years of the experiment were described in detail by Hopkins (1898) and Smith (1908). The analytical methods followed during the middle generations of the study were summarized by Hoener and DeTurk (1938).

During the more recent generations of regular selection, and for the entire period of reverse and "switchback" selection, the experimental material has been grown in a portion of the maize breeding nursery of the Department of Agronomy. The soil is a moderately well-drained, fertile silt loam to silty clay loam. Productivity has been maintained at a moderately high level through crop rotation and the application of limestone and fertilizers in the manner normal for maize production in the area. Until 1952, no supple-

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[2] The author became responsible for field operations and data processing in 1947, and assumed full direction of the project in 1951.

mentary nitrogen fertilizer was applied. In 1952, all strains were grown on land which had received pre-planting nitrogen fertilization at a rate of approximately 90 kg/ha N. From 1953 to 1957, the "oil" strains were grown without supplemental N fertilization, while the "protein" strains were grown both with and without additional nitrogen. Since 1958, all strains have again been grown on land fertilized with approximately 90 kg/ha of supplemental nitrogen.

Sprinkler irrigation was provided during the 1954 and 1959 growing seasons to supplement moisture deficiencies arising from drought. In all other years, the entire supply of moisture for the crop was provided by natural rainfall.

Insofar as possible, only well-filled, fully-developed, and disease-free ears are chosen for chemical analysis. After harvest and drying, 50 to 75 kernels are shelled from each ear. The samples are then ground medium-fine in a laboratory mill, oven-dried, and chemical composition is determined and reported on a water-free basis.

Total nitrogen is determined by the official Kjeldahl-Gunning-Arnold method, slightly modified. Protein percentage is computed by the formula:

$$\% \text{ protein} = 6.25 \ (\% \text{ total N})$$

Oil percentage is determined by extraction of the ground whole-grain samples with Skellysolve F in a Butt apparatus. The extraction time normally is 16 hours[3].

D. RESULTS

I. Degree of Inbreeding

Although the original breeding plan did not contemplate the employment of any special degree of inbreeding, the method of selection which was followed actually resulted in a very rapid narrowing of the ancestral base in each of the four selected populations. Table 1, compiled from data presented by Surface (1913) and Winter (1929), shows the rapid rate at which the majority of the original families were eliminated from the selected populations. As shown by Winter, each of the four selected strains now traces maternally to a single ear in the original population. These "source ears" and their chemical composition are listed in Table 2.

Because male parentage was not controlled and could not be pedigreed, no precise estimate can be made of the inbreeding coefficient at any stage of selection in any strain. Rough computations by the author and his students (unpublished) indicate that the coefficient of inbreeding at least is 50% in each strain, and is more probably on the order of 70 to 80%.

Decreases in yield and vigor in all four selected strains were recognized early in the experiment, and were correctly evaluated as resulting from inbreeding depression. Several modifications, mentioned above, were made in the breeding system in attempts to reduce the degree of inbreeding. These attempts appear to have been relatively unsuccessful until the major change to intra-strain reciprocal crossing was made in 1925. Woodworth *et al.* found grain yields in all four selected strains to be approximately half those

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[3] For many years, the Soil Fertility Division of the Department of Agronomy performed the chemical analyses of the material described in this paper. More recently, these analyses have been carried out by the Service Laboratory of the Department of Agronomy, under the general supervision of Prof. Dr. E. B. Earley.

Table 1. Number of original maternal families represented after selection.

Generations of selection	Selected strain			
	High Protein	Low Protein	High Oil	Low Oil
1	24	12	24	12
2	16	6	16	6
3	15	5	9	5
4	12	5	8	5
5	6	5	8	4
6	3	4	6	4
7	3	3	4	3
8	3	3	4	3
9	2	3	4	3
10	1	2	2	3
11	1	2	2	2
12	1	2	2	1
13	1	2	2	1
14	1	2	1	1
15	1	2	1	1
16	1	2	1	1
17	1	1	1	1
18 *et seq.*	1	1	1	1

Table 2. Source ears and their chemical composition.

Strain	Source ear number	Composition, %
High Protein	H.P. 121	12.28 (protein)
Low Protein	L.P. 106	8.25 (protein)
High Oil	H.O. 111	5.65 (oil)
Low Oil	L.O. 110	4.10 (oil)

of adapted cross-pollinating types of maize. Although obviously not critical evidence, this observation suggests that the level of inbreeding at that time was equivalent to 2 or 3 generations of self-fertilization, or that the inbreeding coefficient was in the range 75 to 87.5%.

II. Regular Selection

The unexpectedly great changes in chemical composition achieved as a result of selection in this experiment have been discussed by Winter (1929), Woodworth *et al.* (1952), and Leng and Woodworth (1953, 1954). As shown in Fig. 1 and 2, and also indicated in Table 3, there is clear evidence that progress in the direction of selection was still occurring in all four strains during the most recent decade of selection. In fact, the response rate in the "High Protein" and "Low Oil" strains appears to have been fully as great in the most recent generations of selection as it was in much earlier stages of the experiment. Progress in the direction of selection in the "Low Protein" and "High Oil" strains has been somewhat slower in recent generations than formerly, but significant progress was also made in both these strains during the most recent 10 generations of selection.

Measurement of the rate and level of response to selection in this material is complicated by the standard of measurement employed (chemical

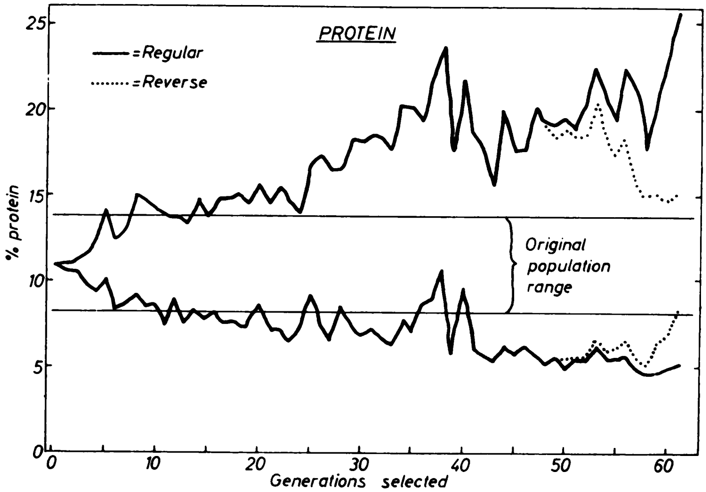


Figure 1. Effects of 61 generations of regular selection and 13 generations of reverse selection on protein content in maize. Range between highest and lowest phenotypes in original Burr White population is indicated as "original population range."

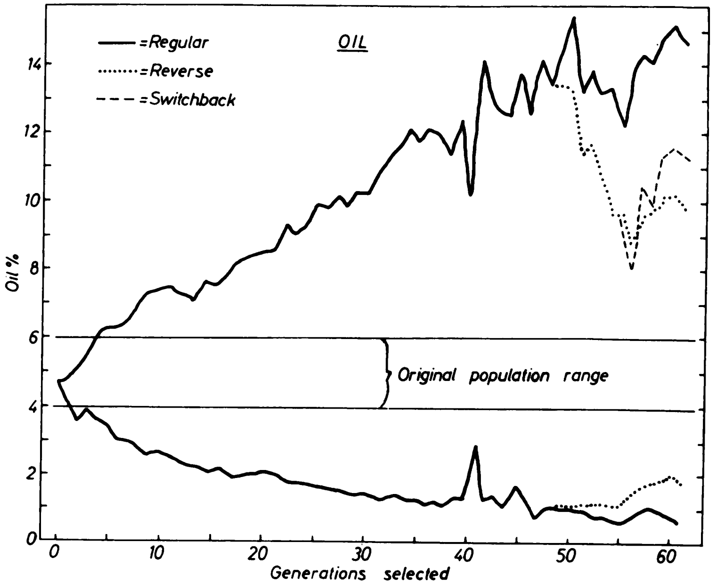


Figure 2. Effects of 61 generations of regular selection, 13 generations of reverse selection, and 6 generations of "switchback" selection on oil content in maize.

Table 3. Progress in altering chemical composition of maize by selection.

Decade of selection	Mean protein, %		Change from previous decade, %	
	High Protein	Low Protein	High Protein	Low Protein
0	10.93			
1	14.42	8.25	+ 41	— 15
2	15.08	7.87	+ 5	— 5
3	18.31	7.05	+ 21	— 10
4	17.95	5.95	— 2	— 14
5	19.53	5.23	+ 9	— 12
6	21.79	4.85	+ 12	— 7

Decade of selection	Mean oil, %		Change from previous decade, %	
	High Oil	Low Oil	High Oil	Low Oil
0	4.68			
1	6.98	2.82	+ 49	— 40
2	8.45	2.04	+ 21	— 28
3	10.42	1.43	+ 23	— 30
4	12.74	1.34	+ 24	— 7
5	13.77	0.96	+ 8	— 28
6	14.83	0.77	+ 8	— 20

composition, expressed in percentage), and also by the familiar difficulty of separating heritable changes from changes resulting from varying environmental conditions. The latter problem is particularly troublesome in assessing response in the "protein" strains, since the level of protein content in cereal grains is well known to be influenced profoundly by environmental conditions such as soil fertility, moisture supply, and length of the growing season. For this reason, it is more satisfactory to evaluate progress by considering average performance during several years than by studying data from individual years. Also, it appears justifiable to eliminate from consideration the data from years where drastic environmental changes be shown to have produced major effects on chemical composition. Therefore, the data in Table 3 reflect 3-year averages at the end of each decade (10-year period) of selection; protein data for the extreme drouth years of 1934 and 1936, and oil data for the years 1936, 1937, 1947 and 1949 are not included in the computations.

Summary of the data by decades, and comparison of the responses from decade to decade, reveal highly interesting trends of response in all four strains (Table 3). These trends may be summarized as follows:

1. High Protein

The mean level of protein content after 60 generations of selection was approximately twice that of the source population mean. Response during the sixth decade of selection was greater than that in any previous period, except the first and third decades of selection.

2. Low Protein

Mean protein content after 60 generations of selection was about 45% that of the source population. Response during the sixth decade of selection was significant, but lower than in any previous decade of selection except the second. Response during the second 30 years of selection, however, was greater than during the first 30 generations.

3. "Protein Strains" Together

The "total range" (Falconer 1960), or difference between the means of the high and low strains after 61 generations of selection, was 19.75% protein, or 19 times the phenotypic standard error of the original population. The difference between the means of the two strains in the 61st generation was the greatest observed during the entire course of the experiment.

4. High Oil

The mean oil content after 60 generations of selection was more than 3 times that of the original population mean, and was approximately 2.5 times as great as the oil content of the highest segregate in the original strain. Response during the last two decades of selection was appreciably slower than during the first 40 generations, but was nevertheless significant, even in the most recent decade.

5. Low Oil

After 60 generations of selection, the mean oil content was only 16% of the original population mean, and was 5 times lower than that of the lowest segregate in the original population. Response during the fifth and sixth decades of selection, measured as percentage change from the respective preceding decades, was fully as great as at any stage of the selection program, except the first 5 to 6 years.

6. Oil Strains Together

The "total range" between means of the High Oil and Low Oil strains in the 61st generation of selection was 14.06% oil, more than 34 times the phenotypic standard error of the original population. The difference between means of the "oil" strains in the 61st generation was the second greatest observed during the course of the experiment, being exceeded slightly only in 1949, when an abnormally high oil analysis occurred in the High Oil strain.

III. Random Mating (Non-Selection)

A brief comparison between the regular selection phase and random mating (non-selection) was made by Woodworth *et al.* (1952), who commented that:

> "in all four strains, the nonselected groups showed some tendency to revert toward the composition of the original Burr White variety."

Reconsideration of the data in their relation to response trends in the selected strains since 1945 leads the present author to the opinion that the conclusion quoted above is erroneous. Therefore, the results of random mating and its comparison with regular selection are again presented (Fig. 3), and will be re-evaluated in their general relation to the effects of continued selection.

1. High Protein

Except in 1934 and 1936, when extreme drouth resulted in the production of abnormally high protein percentages, there was no evidence of any difference between the non-selected and selected populations. Actually, the mean protein content during the last four generations of this study was slightly higher in the random-mated population – 17.81% as compared with 17.72% in the selected group. The difference is non-significant. It may be concluded that neither continued selection nor the relaxation of selection

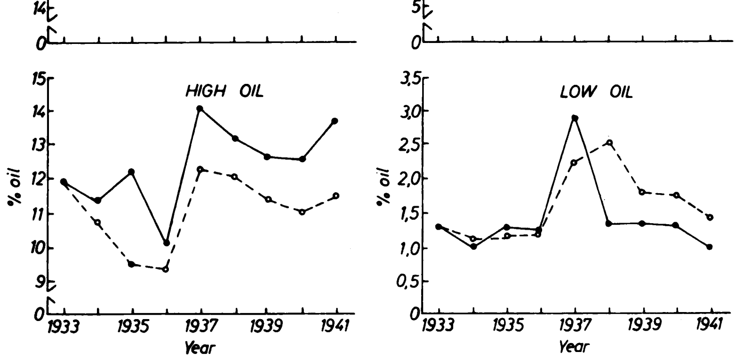


Figure 3. Effects of random mating compared with continued selection for chemical composition in maize. Results from 8 generations of observations, 1934-1941. Solid lines show results of continued regular selection; broken lines refer to random mating.

pressure produced any significant change in protein content during the 8 years of this study. Logically this could be explained as indicating that genes for high protein content were essentially fixed in the High Protein strain in 1933, and that further response to selection should not have been expected. As already indicated, this explanation becomes unsatisfactory when the further response in this strain, during the fifth and sixth decades of selection, is considered.

2. Low Protein

A clear appraisal of the effects of continued selection and random mating in this strain is difficult. At the beginning of this study, rainfall was deficient and summer temperatures were unusually high in three of the first four years. This led to the production of abnormally high protein content, obviously much higher than could normally have been expected in the Low Protein strain. Data from the years 1929 to 1931 indicate that the normal level of protein content in this strain should have been about 7.05% when random mating was begun. If this assumption is correct, 8 generations of random mating resulted in no change, or a slight decline, in protein content. There is no evidence of any "reversion" toward the original population mean. In contrast, the corresponding generations of regular continued selection produced significant progress toward lower protein content. These results are in agreement with conventional expectations and with data obtained in later generations of continued selection in the Low Protein strain.

3. High Oil

The effects of random mating and continued selection were similar to those observed in the Low Protein strain, except that the trends are less obscured by environmentally-induced variations in the data. It is clear that the random-mated population changed little, if any, in mean oil content, while significant progress was made through continued selection in the regular High Oil strain. Here also, the observations are in agreement with theoretical expectations and with the response to later generations of selection.

4. Low Oil

As shown in Fig. 3, the oil content of the random-mated Low Oil population was virtually identical with that of the selected stock during the first four generations of this study. In 1937, a drastic increase in oil content occurred in both the selected and random-mated populations (a similar increase also occurred in the High Oil strains). The selected Low Oil stock reached an oil content of 2.88% – more than twice the level of the immediately preceding generations, and actually higher than at any stage of selection since the 8th generation. In the following year, 1938, oil content in the selected population returned to approximately the previous level, and then declined slightly in subsequent generations of selection. In the random-mated population of Low Oil, the oil content actually increased further in

1938, and remained relatively high in the subsequent years of the "non-selection" study.

The cause of this sudden and drastic change in oil content is not clear, but the behavior of the random-mated stock definitely indicates that the change was heritable in nature. Segregates tending toward higher oil content probably would have been discarded in the course of later selection in the "regular" stocks, but obviously were retained in the random-mated population. These changes could have arisen through accidental contamination of both stocks during the severe drouth in 1936, although there is no actual evidence that this occurred. Also, the increases could have resulted from mutations of major genes controlling oil content.

Unfortunately, seed of the random-mated stock was discarded while the selection experiment was suspended for three years during World War II. Therefore, no further biological evaluation of the events reported above was possible.

5. Effects of Random Mating

Summarizing the comparisons between continued selection and random mating, it is obvious that reversion toward the original population ("regression" in the Galtonian sense) did not occur in any of the four strains. Only in the Low Oil strain was there any significant change in the direction of the original population mean, and in this case, the change clearly resulted from some sudden and drastic genetic alteration which occurred in both the selected and random-mated stocks.

IV. Reverse Selection

Reverse selection was begun to test the existence of genetic variability in the long-selected strains. Analyses by Winter (1929) and Woodworth *et al.* (1952) indicated that total variability, as determined by various statistical measures, had remained essentially unchanged or had actually increased in these strains. The breeding system employed did not permit direct statistical partitioning of variance to estimate its genetic and environmental components. Therefore, it was decided that the actual biological test of reverse selection offered the best opportunity for determining if significant genetic variability remained after 48 generations of selection for chemical composition.

Selection reversal was immediately effective in shifting mean oil content of the High Oil strain back toward the original phenotypic level. Progress in the reverse-selected High Protein and Low Protein strains also became obvious in the first few generations, as pointed out by Woodworth *et al.* (1952) and by Leng and Woodworth (1953, 1954). Reverse selection in the Low Oil strain showed little effect in the first several generations, but then became rapidly and markedly effective. After 13 generations of reverse selection, it is obvious that significant levels of genetic variability have been shown to exist in each of the four long-selected strains (Tables 4 and 5).

Table 4. Results of selection reversal after 48 generations of selection for protein content in maize.

Year	High Protein			Low Protein		
	Regular	Reverse	Difference	Regular	Reverse	Difference
			% protein			
1947	19.24		—	5.11		—
1948	19.14	18.20	— .94	5.50	5.53	.03*
1949	19.45	18.81	— .64	4.91	5.62	.70
1950	18.85	18.42	— .43	5.56	5.64	.08*
1951	20.28	18.67	— 1.61	5.23	5.45	.22
1952	22.52	20.52	— 2.00	6.33	6.54	.21
1953	20.42	18.13	— 2.29	5.53	5.92	.39
1954	19 39	17,42	— 1.97	5.66	6.11	.45
1955	22.36	18.25	— 4.11	5.17	6.60	1.43
1956	21.10	15.95	— 5.15	4.80	5.47	.67
1957	17.80	14.80	— 3.00	4.54	5.08	.54
1958	20.54	15.06	— 5.48	4.79	6.54	1.75
1959	23.04	14.71	— 8.33	4.91	6.76	1.85
1960	24.93	15.09	— 9.84	5.18	8.13	2.95

*) Non-significant difference; all other differences are significant.

Table 5. Results of selection reversal after 48 generations of selection for oil content in maize.

Year	High Oil			Low Oil		
	Regular	Reverse	Difference	Regular	Reverse	Difference
			% oil			
1947	13.45		—	.76		—
1948	14.25	13.45	— .80	1.04	1.10	.06*
1949	15.36	13.32	— 2.04	1.01	1.03	.02*
1950	13.22	11.37	— 1.85	.96	1.13	.17
1951	13.83	11.66	— 2.17	.89	1.13	.24
1952	13.18	10.65	— 2.53	.77	1.15	.38
1953	13.26	9.66	— 3.60	.77	1.27	.50
1954	12.16	9.68	— 2.48	.52	1.04	.52
1955	13.91	8.78	— 5.13	.62	1.36	.74
1956	14.30	9.56	— 4.74	.86	1 67	.81
1957	14.15	9.74	— 4.41	1.02	1.76	.74
1958	14.79	10.16	— 4.63	.91	1.82	.91
1959	15.03	10.31	— 4.72	.76	2.02	1.26
1960	14.66	9.97	— 4.69	.65	1.84	1.19

*) Non-significant difference; all other differences are significant.

1. High Protein

Significant differences between the regular and reverse selected stocks have been observed in every generation of the reverse selection. These differences were relatively small in the early generations of reverse selection. They rapidly became larger, as regular selection resulted in further increases in protein content, while reverse selection brought about definite decreases.

After 13 generations of reverse selection, Reverse High Protein had a mean protein content of approximately 15%, while the regular stock averaged nearly 24% protein in 1959 and 1960. As shown in Fig. 1, the mean protein content of the reverse strain was approximately the same as that in regular High Protein in the 20th generation of regular selection, and was approaching the upper level of the original phenotypic range in the Burr White variety.

2. Low Protein

Differences between reverse and regular selections in Low Protein were non-significant in two of the first three generations. Although statistically significant, these differences remained small until the 7th generation of reverse selection. Each year thereafter, except 1957 (9th generation), there has been clear evidence that reverse seleciton was resulting in a rather rapid increase in protein content. During this same period regular selection appears to have resulted in slight further decreases in protein percentage.

Thus, in the 13th generation of reverse selection, the mean protein content of Reverse Low Protein was about 60% higher than that of the regular selection, and was approximately equal to that of the lowest segregate in the Burr White original population.

3. High Oil

Reverse selection in the High Oil strain has produced large and significant differences in oil content, as compared with regular selection, in each generation. While the regular stock showed little change, or even a decrease, in oil content between 1949 and 1955, the oil content of the reverse selection decreased very rapidly. Since 1955, there has been little change in oil content of the reverse-selected stock, while oil percentage in the regular stock has risen appreciably.

After 13 generations of reverse selection, the Reverse High Oil stock had about 70% as much oil as the regular stock. Oil content of the reverse stock at this time was approximately the same as that in the regular High Oil strain after 30 generations of selection.

4. Low Oil

During the first two generations of reverse selection, the oil content of the reverse stock was virtually identical with that of the regular-selected material. Until the 7th generation of reverse selection, oil content remained almost unchanged in Reverse Low Oil, while declining sharply in the regular Low Oil strain. In succeeding generations, oil percentage in the reverse stock rose very rapidly.

Table 6. Response to first 13 generations of selection, expressed as percentage of phenotypic mean value at start.

Selected strain	Response, % of starting level	
	Regular selection	Reverse selection
High Protein	45	25
Low Protein	20	60
High Oil	55	30
Low Oil	50	100

Reverse Low Oil, after 13 generations of selection, has an oil percentage nearly 3 times higher than that of the regular Low Oil strain, and approximately twice that in the regular strain at the beginning of the reverse selection phase. Considered in relation to the phenotypic levels of the material concerned, progress in Reverse Low Oil has been the most rapid observed in any phase of the entire selection experiment.

5. Rate of Response

Not only the marked effectiveness of reverse selection, but also the rapid rate at which it resulted in significant changes in all four strains is worthy of attention. As shown in Table 6, response in the Reverse Low Protein and Reverse Low Oil strains has been more rapid than initial response to the regular phase of selection, beginning from the original Burr White population. Comparatively, response to reverse selection has been less rapid, but still surprisingly great, in the two "high" strains. Considering the fact that all four strains had been intensively selected for 48 generations, and that each was based maternally on a single open-pollinated ear in the source population, the results of reverse selection lead to the astonishing conclusion that as much genetic variability was present after long-term selection as existed in the original unselected population.

V. "Switchback" Selection

This phase of selection was conducted only in the High Oil strain. Selection was again directed toward higher oil content, after the Reverse High Oil strain had been carried through 7 generations of selection for lower oil content. Results of the first three generations of this selection phase were confusing, but indicated no significant change as a consequence of "switchback" selection. In the fourth generation and subsequently, this type of selection resulted in significant increases in oil content, as compared either with the starting level, or with the current oil content of Reverse High Oil.

Table 7. Oil content in regular, reverse, and "switchback" selections, Illinois High Oil.

Year	Phase of selection		
	Regular	Reverse % oil	Switchback
1954	12.16	—	—
1955	13.91	8.78*	8.02
1956	14.30	9.56	10.40*
1957	14.15	9.74	9.85
1958	14.79	10.16	11.47*
1959	15.03	10.31	11.71*
1960	14.66	9.97	11.47*

*) Significant differences between reverse and "switchback" stocks. All differences between regular selection and others are highly significant.

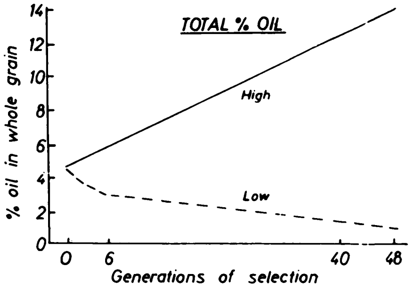
The mean oil content of the "switchback" stock is now approaching the mid-point between that of the reverse and regular High Oil strains (Table 7).

VI. Associated Phenotypic Changes

Marked changes in various phenotypic characters were recognized in all four selected strains during the early generations of selection, as pointed out by Smith (1908). Woodworth *et al.* (1952) summarized and illustrated changes which have occurred in kernel, ear, and plant characters of the selected strains. Perhaps the most significant changes are certain alterations in kernel structure which are clearly correlated with modifications in chemical composition. These were discussed briefly by Woodworth *et al.*, and new evidence regarding the relations with oil content was introduced by Leng and Woodworth (1953, 1954). These changes and their significance may be summarized as follows:

1. Protein Content

An association between endosperm texture and protein content of the kernel was recognized early in the experiment, and was mentioned by Smith (1908). High protein content has been found to be associated with a high percentage of horny endosperm starch, while low protein content is associated with a high soft starch content. At present, the High Protein strain is characterized by small, flinty kernels. The kernels of Low Protein are larger, undented, and show very little horny starch. Although adequate quantitative data are not available, it is apparent from these relationships and from analytical evidence that changes in the zein-rich proteinaceous matrix of the endosperm are primarily involved in the alterations of protein content which have been produced in the two "protein" strains.



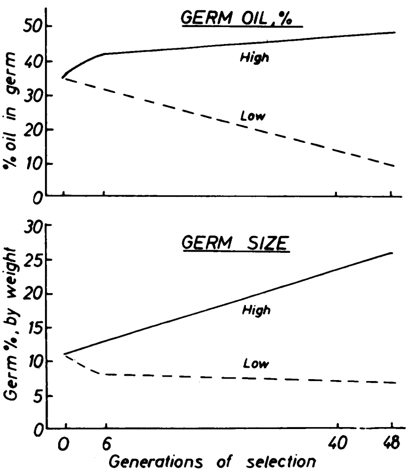


Figure 4. Effects of long-term selection on total oil content and its two major components in maize. Solid lines refer to the "Illinois High Oil" strain and broken lines refer to "Illinois Low Oil."

2. Oil Content

The association between germ size (principally, scutellum size) and oil content of the grain has long been recognized, since the scutellum was shown by Hopkins (1898) to contain most of the oil in the corn kernel. This physical association has been used by Woodworth and Jugenheimer (1948), and no doubt by others, as an easy and practical method for rapidly making selections toward higher oil content.

Separation of oil content into two major components a) germ size, and b) oil content of germ has revealed interesting differences between the High Oil and Low Oil strains, as shown by Leng and Woodworth (1953). As graphically demonstrated in Fig. 4, selection for increased oil content apparently resulted in early fixation of oil percentage in the germ in High Oil. As a result, continued response to selection in this strain resulted primarily from increases in germ size. Selection for decreased oil content in Low Oil resulted in fixation of germ size in a very few generations; continued response to selection in this strain resulted almost entirely from further decreases in oil content of the germ. Thus, the character "oil content" actually can be divided into two components which have responded very differently to selection. A more detailed discussion of this situation, presented in another paper (Leng 1961) points out the significance of these different responses, so far as prediction of future response and estimation of selection limits are concerned.

E. DISCUSSION

In evaluating breeding methods, efficiency frequently is mentioned as a major standard of evaluation. This normally refers to the rate of response, considered in relation to cost factors, labor requirements, elapsed time, and so on. In many respects, effectiveness – the capacity for obtaining a high level of response – is a more important attribute of a successful breeding system. Particularly, the ultimate potential limit of response to selection is of fundamental importance, especially in the most highly-selected, economically important species. Evidence on both efficiency and effectiveness is furnished by data from the long-term selection studies, and will be discussed in the following paragraphs.

Since the majority of quantitative genetic theory is strictly applicable only to idealized situations, it is not surprising to find the results of an actual breeding experiment differing in certain important aspects from theoretical assumptions. However, in several respects, the results of the Illinois long-term selection work differ so widely from normal expectations that some re-evaluation of standard breeding theory appears to be indicated.

Response to long-term selection was asymmetrical, very irregular in rate, and yet reached much greater levels than anticipated, both in protein and oil content. Most surprisingly, great progress toward the objectives of selection in all four selected strains did not lead to decreases in total variability, and reverse selection produced rapid retrograde changes in all strains. These results are especially unusual when considered in connection with the high degree of inbreeding assumed to have occurred.

I. Asymmetry of Response

Falconer (1960) has pointed out that several two-directional selection experiments have shown significant differences in response depending on the direction of selection. Moreover, although listing a number of possible explanations for such asymmetry, he commented that its cause was in most cases unknown.

Asymmetry in response was apparent early in the Illinois selection experiment, and was discussed briefly by Winter (1929) and Woodworth *et al.* (1952). In the "protein" strains, selection for high content produced much more rapid initial progress than selection for low protein. From about the 35th to the 45th generation of selection, little progress was apparent in High Protein, while significant response occurred in the "low" strain. During the most recent 10 to 15 generations, response has been relatively similar in both strains. Asymmetry has been even more pronounced in the "oil" strains, where response to selection for high oil content continued in an essentially straight-line trend for 45 to 50 generations. Response of the Low Oil strain was very rapid in the first few generations of selection, then slowed appreciably. In the most recent generations, the situation appears to have become almost reversed, since the low strain has shown the more rapid relative response to selection.

Falconer (1960) has listed as possible causes of such asymmetrical response the following:

1. Inequality in selection differential.
2. "Genetic asymmetry," either in direction of dominance or in gene frequency.
3. Selection of heterozygotes in one direction.
4. Inbreeding depression.
5. Maternal effects.

He pointed out that examples of actual situations can readily be found which do not fit any of the proposed explanations.

The observed asymmetry of response in the "protein" strains is not readily explained, and no critical evidence is available to aid evaluating its causes. In the "oil" populations, it appears that much of the asymmetry can be explained by considering the phenotypic role of the two major components of oil content, and by evaluating the changes which occurred in these components under selection for total oil content. Leng (1961) discusses this matter in detail, pointing out that germ size in Low Oil was rapidly fixed at its lowest fitness level, thus leaving oil content of the germ as the only component able to respond to selection. In High Oil, relative germ size continued to increase at a nearly constant rate for at least the first 50 generations of selection. Oil content of the germ in this strain responded quickly in early generations of selection, then at a declining rate, apparently tending toward a limit in the vicinity of 50%.

These observations suggest the conclusion that an asymmetry of response to selection may occur if the "character" being selected actually is composed of separate physical or physiological components which react differently and at different rates to opposite directions of selection. If adequate component analysis of such characters could be made, such anomalies as asymmetrical response might more easily be explained.

II. Rate of Response

Attention has already been drawn to the variations and asymmetries observed in rate of response to selection in the different strains. No constant or uniformly-changing rate of response appears to apply to any of the selected strains. The rates of response in this material can be characterized in general as

1. step-wise, rather than steady in rate, and
2. much greater after successful long-term selections than anticipated under any of the usual assumptions about the rate of genetic fixation under selection.

The sustained high rates of response are especially unexpected because of the high level of inbreeding assumed to have occurred in the early generations of the experiment. The continued response must be interpreted as reflecting the existence of significant genetic variability after many generations of selection. This conclusion is supported by the rapid rate of response to reverse selection which was found in all four strains.

III. Level of Response and Selection Limits

Response to continued selection is expected to diminish in rate, and ultimately to cease when all the "favorable" loci have been fixed. The selected population is then considered to have reached a "selection limit". The more intense the level of inbreeding during selection, the more quickly the selection limit is expected to be reached.

Falconer (1960), summarizing the results of four long-term selection experiments – two each in *Drosophila* and the mouse – concluded that:

1. Response continued for 20 to 30 generations.
2. The "total range" (difference between means of "high" and "low" populations) was 10 to 20 times the phenotypic standard deviation in the original population.
3. The total response to selection was less than might be expected.

It has already been emphasized that none of the four long-selected "chemical strains" appears to have reached a selection limit, even after 61 generations of breeding. This is in itself a surprising fact, made more so by the clear evidence that each selected strain originated maternally from a single ear. Moreover, there appears to be little basis for predicting when the selection limit will be reached, except possibly in the Low Oil strain (Leng 1961).

The total range between the two "protein" strains, as mentioned above, was 19.75% protein, or 19 times the original phenotypic standard deviation. In the "oil" strains, the total range at this time was 14.06% oil, more than 34 times the original phenotypic standard deviation. The total response to selection was thus much higher than indicated by 20 to 30 generations of selection in *Drosophila* or mice, and apparently could increase significantly under further selection.

It is especially noteworthy that the levels of oil and protein content achieved as a result of long-term selection were far higher (or lower, respectively) than any known to exist in normal populations of dent maize. For these characters, at least, the breeding methods used have created phenotypic levels of expression quite unknown in the species. Yet, genetic fixation of the selected characters has not resulted.

IV. Retention of Genetic Variability

The failure of total variability to decrease in the selected strains was clearly shown by Winter (1929), who however apparently considered that much of the "retained variability" was non-genetic in nature. Subsequent observations, especially the rapid and marked response to reverse selection, have made it clear that all four strains possessed significant levels of genetic variability after 48 generations of selection. Present evidence indicates that genetic variability still remains after 60 generations of selection.

This fact, more than any other of the observations made in this experiment, stands in direct contradiction to the conventional expectations in selection. Falconer (1960) has discussed the retention of variability in long-selected populations, suggesting these possible explanations:

1. An increase in environmental (non-genetic) variance.
2. Retention of heterozygosity through the action of natural selection for fitness.
3. Favoring of heterozygotes through artificial selection, or a combination of natural and artificial selection.

Either or both of the latter two situations could be operating in the long-selected "chemical strains". However, comparison of the rate of progress under reverse selection with response rates in the early generations of regular selection leads to the conclusion that mean levels of oil and protein content were shifted drastically by selection with no decrease in heterozygosity of the genes controlling these characters. Yet, such a conclusion scarcely seems reasonable, and cannot readily be reconciled with conventional theory on genetic control of quantitative characters.

V. Possible Genetic Interpretations

It therefore remains necessary to look for genetic interpretations which fit the patterns of response to long-term selection observed in this study. Possible reasons for the continued existence of genetic variability in long-selected material include:

1. Accidental outcrossing (contamination).
2. Favoring of heterozygotes in selection.
3. A high mutation rate of the genes controlling the characters under selection.
4. Release of genetic variability through some mechanism other than those described above.

The first of these explanations can essentially be dismissed from consideration in this study. Technical control of purity in pollination has been strict, at least since ear-to-row selection was discontinued. The "oil" strains are practically isolated from crossing with each other and with other strains, because High Oil flowers very early and Low Oil very late. Most important, a biological control of purity is provided by the fact that all four "chemical" strains have white endosperm (recessive), while all other maize in the surrounding nursery carries the dominant yellow alleles. Thus, any contamination by pollen from surrounding maize can be detected when the ears are examined at harvest, and ears showing such contamination are always discarded.

Favoring of heterozygotes in selection has already been mentioned as an unsatisfactory explanation for the observed results of long-term selection in this material. The rapid response to reverse selection in all four strains, if it were attributed to residual heterozygosity alone, would have required the level of heterozygosity to have remained at nearly the same level through 48 generations of successful selection. This appears highly improbable. Even more important, genetic variability was demonstrated in both the "high" and "low" stocks, for both oil and protein content. Retention of heterozygosity under selection is theoretically possible in unidirectional selection, but it is unreasonable to expect the degree of retention to be high in both directions when a trait is selected for opposite extremes.

The recent findings of Sprague *et al.* (1960) that appreciable mutation rates exist for genes controlling some economic characters in maize may be interpreted as evidence that mutation could provide an important source of continued variability in the "chemical strains". Attempts by the present author and his students to estimate mutation rate in the High Oil strain have thus far been unsuccessful (unpublished). However, there appears to be no evidence that the rate of mutation in genes controlling protein and oil content is significantly higher than the order of 10^{-4} to 10^{-8}, reported by Falconer (1960) as being those normally accepted. So far as physical characteristics of the selected strains are concerned, all four strains are relatively uniform and show no evidence of being highly mutable. Thus, mutation is not considered a likely explanation for the apparently high level of genetic variability remaining in the long-selected strains.

By exclusion, some other mechanism producing continued release of genetic variability must be considered the most logical explanation for the long-continued response to selection with retained genetic variability. Dobzhansky *et al.* (1959) have discussed a mechanism in *Drosophila prosaltans* which leads to continued release of genetic variability through recombination. The existence of such a situation in other organisms, as for example maize, must be considered possible. Unfortunately, no critical evidence is presently available to test the assumption that such a mechanism has operated in the long-selected maize strains. Yet, the fact that the breeding program employed has involved selection and continual recombination within a relatively small, close-bred population suggests that recombination must in some manner have played an important role in the response obtained. A more detailed and critical consideration of this possibility appears to be highly important.

VI. Application to Breeding Theory

The prediction of response to selection and the evaluation of breeding systems are based on extrapolation from observed trends to anticipated future events. In the experiments reported in this paper, even 61 generations of observations do not appear sufficient to permit accurate prediction of response to continued selection. Yet, it appears reasonable to assume that further response can be expected in all four long-selected strains.

In evaluating the desirability of different breeding systems, it is necessary to distinguish between early response rate and the possibility of obtaining the highest maximum response. Most current evaluations of breeding methods are based on short-term results, which often may not reflect the actual long-term potentialities. Especially where a species or population shows evidence of approaching a selection limit for a desired character – as may for example be true of grain yield in maize – breeding systems which offer possibilities of obtaining response without appreciable loss of genetic variability merit particular attention.

The high level of response obtained and its long duration in the Illinois selection experiments suggest that breeding systems involving frequent recombination may be especially effective in obtaining maximum levels of phenotypic expression before a selection limit is reached. Such selection apparently can be conducted even in relatively small, closed populations without excessive loss of genetic variability. These observations lead to the conclusion that, where high ultimate levels of phenotypic expression are desired, breeding systems emphasizing recombination are preferable to those leading to rapid genetic fixation.

SUMMARY

Selection for both high and low levels of total protein and oil percentage in the grain has been conducted in maize for 61 generations. Despite a relatively high apparent level of inbreeding, response to selection has persisted in all four strains, and further response appears to be possible.

Phenotypic mean values in all four selected strains have been completely outside the phenotypic range of the original population since the first few generations of selection. After 61 generations of selection, the total range between the high and low strain means was about 19 times the original standard deviation in the "protein" strains, and more than 34 times the original phenotypic standard deviation in the "oil" strains.

Response to selection has occurred in an irregular, stepwise manner, rather than at a uniform rate. Thus, prediction of response trends has been difficult and inaccurate.

Total variability has remained at unexpectedly high levels in the selected strains. Rapid and significant response to reverse selection have indicated that high levels of genetic variability were present in all four strains after 48 generations of selection in the original program.

Important questions about the nature of genetic changes under long-term selection are raised by the rapid rate and high levels of response to selection in this material. Accidental outcrossing, heterozygote superiority, and high mutation rate have been evaluated as causes of the continued existence of significant genetic variability under long-term selection pressure. None of these situations appears to offer an adequate explanation of the observed results.

Continued release of genetic variability through recombination, possibly assisted by favorable mutations and some retention of heterozygosity, is suggested as the primary mechanism involved in continued response to selection under the breeding system employed. Unfortunately, critical evidence is not presently available to test this assumption.

The high level of response obtained, and the long duration of response, suggest that breeding systems which permit continued recombination during many generations may be especially effective in obtaining maximum levels of expression in desired characters.

EDITOR'S NOTE

This paper was based on a lecture series presented at the Max-Planck-Institut fur Zuchtungsforschung, Koln-Vogelsang, during tenure of a Fulbright Senior Research Fellowship, in 1961. At that time the author was Professor of Plant Breeding and Genetics, College of Agriculture and Illinois Agricultural Experiment Station, University of Illinois. The paper was dedicated to Dr. W. Rudorf, who suggested its preparation and presentation, and to the memory of the late Dr. C. M. Woodworth, "whose keen scientific judgement was responsible for continuity in the experiment during many years when its value was not generally appreciated." A German summary, printed with the original text, has been deleted.

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8

Genetic Variability After 65 Generations of Selection in Illinois Oil and Protein Strains of Zea mays *L.*

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ABSTRACT

Significant genetic variability, as measured by variation among half-sib family means, was found for both oil and protein content after 65 generations of selection in Illinois high oil (IHO), low oil (ILO) high protein (IHP) and low protein (ILP) strains of maize (*Zea mays* L.). Correlated responses between oil and protein over the 65 generations of selection have been small. Predicted gains per generation based on selection of the upper 20% of half-sib families from a test of one replicate grown at one location in 1 year were slightly higher than those observed as an average of 65 generations for IHP and ILP and lower for IHO and ILO.

Additional index words: Correlated response, Heritability.

INTRODUCTION

Periodic reports on the changes occurring in the Illinois long term selection experiments have been made [Hopkins (1899), Winter (1929), Woodworth, Leng and Jugenheimer (1952), and Leng (1962)] . Through 1965, selection had been continuous for the character and direction indicated in the Illinois high oil (IHO), low oil (ILO), high protein (IHP) and low protein (ILP) strains of maize (*Zea mays* L.) for 65 generations. Leng (1962) demonstrated the existence of residual genetic variability after 48 generations in all four strains by reverse selection experiments. However, no attempt was made to measure the relative magnitude of the genetic variance remaining in the populations.

The objectives of this study were: (1) to determine whether significant genetic variability for oil and protein content still exists in IHO, ILO, IHP, and ILP, (2) to determine the relative magnitude of such genetic variability if it existed, (3) to measure the correlated response between oil and protein in the four populations which occurred during 65 generations of selection, and (4) to predict possible future gains from selection.

MATERIALS AND METHODS

Leng (1962) gave a detailed description of the selection procedures used in developing the IHO, ILO, IHP, and ILP populations. Seed for this study came from the 1965 crop of 60 ears analyzed from each population and represent the populations at the end of 65 generations of selection. In 1966, remnant seed from each of the 60 ears from each population was used to establish four experiments of 60 half-sib families each to measure the magnitude of any residual genetic variability. The four experiments were grown in the same field on the Agronomy South Farm, Urbana, Ill. Each test consisted of three replications of single row plots, 10 plants long with 1 m between rows and .33 m between plants within a row. In 1967, remnant seed from the same ears was used to establish four identical experiments plus a test of IHP to which an additional application of 280 kg/ha of nitrogen (N) (applied as a complete fertilizer in a 2:.83:.44 ratio of N:P:K) was made approximately 3 weeks prior to silk emergence. In each experiment the 60 ears were assigned at random to four blocks of 15 entries each. Blocks were randomized within replications and ears were randomized within blocks. At pollinating time pollen from plants grown from the bulked, remnant seed of the 60 ears of each experiment was collected in bulk and used to pollinate five or six plants in each row of the corresponding experiment. At harvest seed from the center portions of four well filled ears resulting from such pollinations were bulked for chemical analyses of each plot.

Oil content of a 25-g sample from each plot was determined by nuclear magnetic resonance spectroscopy as described by Alexander et al. (1967). Protein content was determined by the official Kjeldahl-Gunning-Arnold method, slightly modified.

Because of poor stands in the ILO population no data were obtained in 1967. In the ILP population only 31 families were available for a combined analysis over both years because of poor stands in 1967. The IHO and IHP populations had 58 and 55 families, respectively, included in the combined analysis over both years. Because of the missing families the combined data were analyzed as a randomized complete block grown in 2 years. Years and families were considered random in estimating components of variance.

RESULTS AND DISCUSSION

The original Burr White population in 1896 had 10.9% protein and 4.7% oil (Hopkins, 1899). The data in Table 1 represent the largest number of analyses, in a given year, of each population since the beginning of the experiment and indicate a gain of 14.3 percentage points protein in IHP, a loss of 5.7 percentage points protein in ILP, a gain of 10.5 percentage points oil in IHO, and a loss of 4.3 percentage points oil in ILO.

Oil content was slightly higher and protein content slightly lower for all three populations in 1967 than in 1966 (Table 1). Means in Table 1 include only families for which data were available from both years where 2 years' data are shown.

Table 1. Mean oil and protein contents of Illinois long-term chemical selections grown in 1966 and 1967.

Population	Oil, %			Protein, %		
	1966	1967	Avg	1966	1967	Avg
Illinois high oil	15.0	15.4	15.2	15.5	13.1	14.3
Illinois low oil	.4	---	---	12.8	---	---
Illinois high protein	5.4	6.1	5.7	25.9	24.5	25.2
Illinois low protein	3.2	3.4	3.3	5.2	5.1	5.2
Illinois high protein (high N)	---	6.1	---	---	25.6	---

Table 2. Estimates of components of variance for oil and protein content from Illinois long-term chemical populations.

Population	Oil			Protein		
	σ_g^2†	σ_{gy}^2†	σ^2 †	σ_g^2	σ_{gy}^2	σ^2
Illinois high Oil	.0724*	.0075	.5007	.1082*	.0538	.7891
Illinois low oil	.0008*	--- ‡	.0039	.0859**	---	.2243
Illinois high protein	.0438**	.0029	.0632	.1726**	.0275	.6434
Illinois low protein	.0090*	-.0031	.0522	.0538*	.0268	.1535
Illinois high protein (high N)	.0210**	---	.0491	.3136**	---	.8888

* and ** indicate components significant at the .05 and .01 probability levels as measured by F test. †σ_g^2 = progeny components; σ_{gy}^2 = interaction of progeny component by years; σ^2 = error component of variance. ‡ No measure of genotype X year interaction because data were obtained from ILO only in 1966 and from IHP (HN) only in 1967.

The correlated responses that have occurred are of particular interest. Average oil content of IHP and ILP is 4.5%, not greatly different from the 4.7% of the original population. However, oil content in IHP increased 1.0 point while it decreased 1.4 points in ILP. Thus, drastic alteration of protein content has had only a minor effect on oil content. The protein content of both IHO and ILO is higher than in 1896. However, IHO is 2.7 points higher in protein than ILO. The increase in protein content of ILO since 1896 may be due to increased N fertilization rather than to an effect of selection. It seems reasonable to assume that the 2.7 point difference in protein content between IHO and ILO in 1966 reflects the results of selection. This difference is quite comparable to the 2.4 point difference in oil content between IHP and ILP. Although the absolute changes in oil content in IHP and ILP were similar the differences per unit change in protein content were quite dissimilar. In IHP, each percent increase in protein was accompanied by .07-point increase in oil while in ILP each percent reduction in protein content was accompanied by a reduction of .25 in oil percent. Thus, the magnitude of the correlated response varied with the direction of selection.

From these results it is apparent that altering either oil or protein content in an ordinary maize selection program is unlikely to lead to major changes in the other character.

Estimates of $\sigma_g{}^2$ the progeny component of variance were significant for all five populations for both oil and protein (Table 2) indicating that detectable genetic variability still exists after 65 generations of selection. The genotype X year interaction (σ_{gy}^2) was small and nonsignificant in all cases where it could be measured. The progeny component represents the covariance of half sibs but cannot be interpreted in terms of a fraction of the additive genetic variance because the degree of inbreeding and state of linkage disequilibrium are unknown. Direct comparisons of σ_g^2 between characters and populations cannot be made because of varying magnitudes of error variance. However, the ratio of σ_g^2 to ($\sigma_g^2 + \sigma_{gy}^2 + \sigma^2$) a rough measure of heritability on a single plot basis, should be comparable between characters and tests (Table 3). Heritability for the non-selected characters in each population was expected to be higher than for the selected characters since the only force restricting genetic variability would be inbreeding while for the selected characters both inbreeding and selection were operating. The small correlated response observed reinforced this expectation. For ILO and IHP heritability for the non-selected character was higher than for the selected. However, there was little difference in the heritability of oil and protein in the IHO and IHP (HN) populations while in ILP heritability for protein was higher than for oil.

Heritability for oil content in ILO was slightly higher than in IHO. However, it is doubtful if the observed variability in oil content in ILO is usable for continued selection toward low oil since observation indicated that plots varied in percent germless kernels. In maize, oil is found primarily in the germ. Thus variation in percent germless kernels would increase the genetic variability for oil content but the increased variability would not be usable because germless kernels could not be propagated. An additional indication that oil content in ILO has reached a lower limit comes from the experience in 1967 in the regular selection from ILO in which the lowest oil ears could not be used because of a high percent of germless kernels and poor germination of kernels having germs.

The purpose of including the high nitrogen treatment in IHP (HN) was to determine whether genetic variability for protein content might be increased under a higher N regime. In theory, gene combinations might have accumulated that were limited in their phenotypic expression of protein content because of the level of soil N. Heritability for protein content was slightly higher in IHP (HN) than in IHP (Table 3), and the mean was increased (Table 1). The range in 1967 for IHP was 3.0% while for IHP (HN) it was 3.2%, values that are not greatly different. However, the genotype by nitrogen level interaction was highly significant. Examination of the family means indicated that only five families were included in the top 12 of both IHP and IHP (HN). In addition, seven families had lowered protein contents in the IHP (HN) experiment than in the IHP experiment. Thus the general effect on IHP families of increasing N level was to increase protein content in the lower protein families more than in the higher protein families. A similar, and more striking genotype X N level interaction occurred for oil content. In fact the lowest oil family (5.6% oil) and the highest oil family

Table 3. Ratios (in percent) of σ_g^2 to phenotypic variance of a plot for oil and protein content.

Population	Oil	Protein
Illinois high oil	12.5	11.4
Illinois low oil	16.7†	27.7†
Illinois high protein	39.8	20.5
Illinois low protein	15.5	23.0
Illinois high protein (high N)	30.0†	27.3†

† Based on data from only one year.

Table 4. Average gain per generation (65 generations) and predicted gain at end of 65 generations for the selected character in long term chemical populations.

	Gain per generation†	
Population	Actual	Predicted‡
IHO	.16	.13
ILO	-.07	-.02
IHP	.22	.26
ILP	-.09	-.16
IHP (HN)	---	.42

† Values are percent oil for IHO and ILO and percent protein for IHP, ILP and IHP (HN). ‡ Based on selection among plot means from 1 year, 1 location, 1 replication using the formula $G = K\sigma_p H$ where $K = 1.4$ (selection differential when the upper 20 percent of families are selected); σ_p = phenotypic standard deviation; and H = heritability values from Table 3.

(6.8% oil) in the IHP experiment had nearly equal oil contents (6.3 and 6.2%, respectively) in the IHP (HN) experiment. There was a general tendency for families showing increases in oil content with increased N to show reductions or very small increases in protein content.

The presence of significant genetic variability in the long term populations indicates that additional progress should be possible in all except the ILO population where a selection limit may have been reached. The heritability values reported apply strictly to selection based on a plot basis. Because the basic design of the classical selection experiments depends on selection among individual ears predicted progress would be less than is indicated by the heritabilities reported here.

Actual average change per generation (based on 1966-67 means and original population values) for 65 generations in the selected character ranged from -.07% oil in ILO to .22% protein in IHP (Table 4). Predicted changes based on the heritability values in Table 3 and selection of the upper 20% of families ranged from -.02 for ILO to .26 for IHP (Table 4). The predicted gain in IHP and ILP on this basis is slightly higher than the average gain actually achieved over 65 generations while it is lower for IHO and ILO.

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9

Seventy Generations of Selection for Oil and Protein Concentration in the Maize Kernel

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INTRODUCTION

Seventy generations of divergent selection for both oil and protein concentration in the maize (*Zea mays* L.) kernel were completed with the 1969 crop. The experiment was initiated in 1896 by C. G. Hopkins to determine whether chemical composition of the corn kernel could be altered by selection. In 1908, L. H. Smith listed a practical objective for each strain in the selection program:

1. Increasing percent protein is desirable because corn does not contain sufficient protein for most feeding purposes.
2. Decreasing percent protein is desirable for manufacturers of products derived from the starch in corn.
3. Increasing percent oil is desirable because corn oil is, pound for pound, the most valuable constituent of the grain in commercial use.
4. Decreasing percent oil is desirable for feeding purposes because corn oil tends to produce soft fat in pork.

Although the four strains, Illinois high protein (IHP), Illinois low protein (ILP), Illinois high oil (IHO), and Illinois low oil (ILO), have not been used directly as commerical varieties, germplasm from the strains has been used commercially. The first apparent practical use of germplasm from this experiment was in the widely used double cross hybrid, the Double-Crossed

Burr-Leaming. One of the single-cross parents of this hybrid was produced by crossing an inbred line from IHP with one from ILP.

In the 1940's, at the Illinois Agricultural Experiment Station, high protein lines developed by crossing standard inbred lines to IHP and backcrossing to the standard inbred parent were tested in hybrid combinations (Jugenheimer, 1958). Because yields were low and the increased protein was of poor nutritional quality, the lines never became commercially important. In the 1960's, inbred lines higher in oil were developed using IHO as a genetic source for increased oil percent. Some of these lines are being used commercially on a small scale.

Information on the nature of response to selection, which the accumulated data can provide, is as important as the use of the strains as germplasm sources. Thus, this chapter will present a comprehensive summary and interpretation of the direct and correlated responses to 70 generations of divergent selection for both oil and protein concentrations in the maize kernel, evaluate and interpret results of reverse selection, and examine the results of supplementary experiments designed to verify the results observed in the main selection experiment.

MATERIALS AND METHODS

Breeding Procedures

A detailed description of the selection procedures and analytical methods used in in the first 10 generations was given by Smith (1908). Winter (1929) described the breeding procedure through 28 generations. Leng (1962a) summarized the breeding and analytical procedures used through the first 60 generations. A summary of these procedures is included here to aid interpretation of the results.

The population selected for study was the open-pollinated variety, 'Burr's White.' Seed of Burr's White were obtained from Mr. F. E. Burr, Champaign County, Illinois in 1887. The variety was maintained by the Illinois Agricultural Experiment Station until selection was initiated (Smith, 1908). In 1896, 163 open-pollinated ears (.6 hl = 2 bu) were analyzed for percent oil and percent protein. After analysis the 24 ears highest in protein, the 12 ears lowest in protein, the 24 ears highest in oil, and the 12 ears lowest in oil were selected to initiate the Illinois high protein (IHP), low protein (ILP), high oil (IHO), and low oil (ILO) strains, respectively. Seed from the selected ears of each strain were planted ear-to-row in a separate isolated field. Through generation 9, the number of ears analyzed and selected each generation varied (Table 1). However, approximately equal numbers of ears from each row were analyzed and the most extreme ears were saved without regard to the row from which they came. In generation 10, the system was modified in an attempt to reduce the amount of inbreeding. Alternate rows were detasseled and four ears from each of the six highest yielding detasseled rows were selected. In generation 25, the system was changed to select 2 ears from each of the 12 detasseled rows. In generation 29, the number of ears analyzed was reduced to 60 and a system of intrastrain crossing was introduced. Twelve selected ears from each strain were arbitrarily divided into two lots, A and B, of six ears each. Seed within each lot were bulked and planted in the nursery. Pollen from several plants (15 to 20) in lot A was bulked and used to pollinate several plants in lot B. Similarly, pollen from B was used to pollinate plants in A. After harvest, 30 ears with good seed-set from each lot were analyzed. Of the 60 ears analyzed within a strain the 12 more extreme ears were selected. This procedure is still being used.

Table 1. Number of ears analyzed and selected each generation in IHP, ILP, IHO, and ILO strains.

Generation*	IHP		ILP		IHO		ILO	
	Analyzed	Selected	Analyzed	Selected	Analyzed	Selected	Analyzed	Selected
0	163	24	163	12	163	24	163	12
1	112	24	48	12	80	24	40	12
2	252	24	126	16	216	12	108	16
3	216	24	135	16	108	12	144	16
4	216	15	144	14	108	14	144	14
5	132	14	125	14	125	14	125	14
6	90	22	90	22	90	22	90	22
7	100	22	100	22	100	22	90	22
8	100	25	100	25	101	25	100	25
9	119	24	120	24	120	24	119	24
10 to 28	120	24	120	24	120	24	120	24
Except for:								
12	119							
13		25		23				25
29 to 70	60	12	60	12	60	12	60	12
Except for:								
34							38	
38	34						43	
40							50	
46	80		38		56		31	
59		10		10		10		10
60							57	
64	59							
67	57		52					
68	58		58					
69			57					
Total	6287		5916		6007		5842	

* Except for generations and strains indicated the number of ears analyzed and selected is the same for all generations and strains.

The forward selection phase of the experiment, therefore, can be divided into four segments based on the breeding procedures used and the generation when nitrogen fertilizer was first added as follows: 1) generations 0 to 9, selection based only on chemical composition; 2) generations 10 to 25, selection based on yield and chemical composition; 3) generations 26 to 52 in IHP and ILP and generations 26 to 58 in IHO and ILO, intrastrain crossing with selection for chemical composition and no added N; and 4) generations 53 to 70 in IHP and ILP and generations 59 to 70 in IHO and ILO, intrastrain crossing with selection for chemical composition and the addition of 90 kg/ha of N.

In generation 48, reverse selection was initiated in each of the four strains (Leng, 1962b). The procedure was identical to that in the forward selection experiment except that selection was for low protein in the high protein strain, high oil in the low oil strain, etc. Thus, four new strains were formed: reverse high protein (RHP), reverse low protein (RLP), reverse high oil (RHO), and reverse low oil (RLO). In the initial generation of reverse selection only six ears were saved in each strain (Table 2). Thereafter, with 1 exception, 12 ears were saved each generation. After 7 generations of selection in RHO, a new strain, designated switchback high oil (SHO), was initiated by selecting the 12 ears in RHO that were highest in oil (Leng, 1962b).

Table 2. Number of ears analyzed and selected each generation in RHP, RLP, RHO, and RLO strains.

Generation*	RHP		RLP		RHO		RLO	
	Analyzed	Selected	Analyzed	Selected	Analyzed	Selected	Analyzed	Selected
0	60	6	60	6	60	6	60	6
1 to 22	60	12	60	12	60	12	60	12
Except for:								
5			51				45	
6			59		59			
8			49				50	
11		10		10		10		10
19	54							
20			54					
21	59							

* Except for generations and strains indicated the number of ears analyzed and selected is the same for all generations and strains.

The reverse phase of the experiment was divided into two phases based on the date of application of N as follows: 1) generations 0 to 4 in RHP and RLP and generations 0 to 10 in RHO and RLO; and 2) generations 5 to 22 in RHP and RLP and generations 11 to 22 in RHO and RLO.

Statistical Analysis

In each generation, seed from each ear in a particular strain were analyzed for the selected trait (e.g., protein percentage in IHP). At the same time, a bulk sample of seed of all ears was analyzed for the nonselected trait (e.g., percent oil in IHP). Thus, for each generation of each strain, individual ear data are available for the selected trait and an average value is available for the nonselected trait. In addition, the selected ears were identified. From these data the following statistics were calculated for each generation: mean of the selected character ($\bar{x}$), mean of the selected ears ($\bar{x}_s$), selection differential ($\bar{x}_s - \bar{x}$), variance, standard deviation (S. D.), and coefficient of variation (C. V.).

Because of the small difference in protein percentage between IHO and ILO, even after 70 generations of selection (Table 3), and the large year-to-year fluctuation in percent protein, the mean protein values for IHP, ILP, RHP, and RLP for a particular year were adjusted by subtracting the mean percent protein of IHO and ILO for that year. Adjusted protein values were used to calculate realized heritabilities and correlated response. Oil values from the oil strains were not adjusted because of low year-to-year fluctuation and an apparent decline in percent oil in ILP.

Realized heritabilities for each strain were calculated from regression of generation means on cumulative selection differential for each of the four segments of the experiment. The procedure of dummy variables in multiple regression, as described by Draper and Smith (1966), was used. This procedure assumes a linear relationship between the mean and cumulative selection differential within each segment and that progress in each succeeding segment starts from the point where progress in the preceding segment stopped. Estimates of additive genetic variance for segments 1, 3, and 4 were calculated by multiplying twice the average total phenotypic variance for each segment times the realized heritability for the segment. For segment 2, additive genetic variance was calculated as 2.6667 times the average within-row phenotypic variance times the realized heritability. This procedure adjusts for differences in breeding procedures between segment 2 and the other segments but does not adjust for inbreeding. Correlations of means with

Table 3. Data from 70 generations of selection in Illinois High Oil and Illinois Low Oil.

	Illinois High Oil					Illinois Low Oil				
	Mean % Oil					Mean % Oil				
Generation*	All Ears	Select Ears	S.E.†	C.V.	% Protein	All Ears	Select Ears	S.E.†	C.V.	% Protein
0	4.69	5.33	.42	9.0	10.9	4.69	4.04	.42	9.0	10.9
1	4.79	5.20	.38	7.9	10.8	4.10	3.65	.39	9.4	11.0
2	5.10	6.15	.48	9.3	---	3.95	3.47	.33	8.3	---
3	5.65	6.30	.43	7.6	---	3.85	3.33	.33	8.5	---
4	6.10	6.77	.45	7.3	10.8	3.57	2.93	.36	10.2	11.0
5	6.24	6.95	.45	7.3	12.3	3.45	3.00	.26	7.7	10.0
6	6.26	6.76	.52	8.3	10.8	3.00	2.62	.33	11.0	9.3
7	6.51	7.15	.48	7.3	11.0	2.99	2.90	.23	7.8	10.2
8	7.12	7.73	.58	8.1	12.3	2.91	2.66	.25	8.5	10.9
9	7.29	7.92	.55	7.5	12.1	2.58	2.30	.27	10.4	9.9
10	7.37	7.90	.45	6.1	11.8	2.66	2.29	.31	11.7	10.5
11	7.43	7.98	.47	6.3	10.9	2.59	2.40	.22	8.6	9.2
12	7.20	7.90	.56	7.7	11.2	2.39	2.07	.26	10.6	10.9
13	7.04	7.68	.48	6.8	10.4	2.23	2.06	.25	11.1	10.3
14	7.72	8.33	.51	6.6	10.2	2.20	1.90	.24	11.1	10.0
15	7.52	8.27	.68	9.0	10.2	2.05	1.83	.22	10.7	10.1
16	7.71	8.26	.50	6.5	11.1	2.18	1.84	.26	12.1	9.8
17	8.15	8.98	.64	7.9	11.4	1.90	1.66	.24	12.9	10.5
18	8.30	9.04	.64	7.7	11.6	1.98	1.79	.26	13.0	9.8
19	8.46	9.11	.57	6.8	10.1	2.07	1.84	.24	11.7	10.4
20	8.50	9.18	.57	6.7	11.3	2.06	1.70	.50	24.1	11.2
21	8.52	8.08	.54	6.4	9.7	2.09	1.85	.25	11.8	9.0
22	9.35	9.97	.53	5.7	11.4	1.87	1.60	.30	16.1	9.2
23	9.06	9.77	.68	7.5	10.3	1.77	1.54	.20	11.6	8.5
24	9.28	9.98	.52	5.6	---	1.79	1.49	.22	12.3	10.3
25	9.94	10.67	.62	6.3	12.8	1.69	1.42	.26	15.5	11.4
26	9.85	10.46	.52	5.2	12.2	1.67	1.37	.23	13.8	10.2
27	10.08	10.91	.63	6.3	12.5	1.58	1.35	.25	15.8	10.8
28	9.86	10.61	.69	7.0	12.0	1.51	1.24	.23	15.2	11.1
29	10.21	11.02	.63	6.2	12.2	1.43	1.08	.28	19.8	11.0
30	10.21	11.20	.73	7.1	10.8	1.43	1.07	.27	18.7	11.2
31	10.85	11.62	.61	5.6	11.3	1.42	1.13	.23	16.4	10.4
32	11.25	12.09	.59	5.3	12.0	1.29	1.11	.24	18.3	13.9
33	11.52	12.24	.61	5.3	12.3	1.36	1.17	.19	13.7	10.2
34	12.10	12.91	.65	5.4	13.6	1.28	1.18	.20	15.4	13.5
35	11.80	12.53	.67	5.7	13.8	1.25	1.06	.22	17.4	12.6
36	12.04	12.85	.63	5.2	15.0	1.18	.88	.23	19.4	12.4
37	11.97	13.14	.92	7.7	14.8	1.27	1.05	.21	16.5	13.3
38	11.36	12.76	1.19	10.5	16.0	1.04	.93	.20	19.5	16.8
39	12.48	13.65	.77	6.2	---	1.32	.95	.28	21.5	---
40	10.14	11.32	.91	9.0	17.6	1.22	.87	.27	21.9	13.8
41	14.11	15.47	.94	6.7	12.6	2.88	2.50	.28	9.6	12.2
42	13.14	14.24	.80	6.1	12.7	1.34	1.11	.17	12.6	11.4
43	12.61	13.74	.85	6.8	11.9	1.37	1.13	.19	13.6	10.2
44	12.57	13.58	.69	5.5	12.9	1.36	1.05	.27	20.0	12.6
45	13.73	14.74	.90	6.6	13.7	1.02	.78	.22	21.2	10.6
46	12.62	13.71	.93	7.4	---	1.53	1.15	.51	33.7	---
47	14.12	15.64	1.04	7.4	13.1	1.21	.94	.21	17.5	12.1
48	13.45	14.61	.92	6.9	12.5	.76	.54	.16	20.5	12.2
49	14.25	15.30	.78	5.4	13.5	1.04	.75	.20	19.4	11.6

(Continued)

Generation*	Illinois High Oil					Illinois Low Oil				
	Mean % Oil				% Protein	Mean % Oil				% Protein
	All Ears	Select Ears	S.E.†	C.V.		All Ears	Select Ears	S.E.†	C.V.	
50	15.36	16.60	.90	5.9	12.8	1.01	.77	.17	17.0	11.2
51	13.23	14.69	1.12	8.4	13.1	.96	.76	.18	18.6	11.2
52	13.83	15.03	.86	6.2	14.4	.89	.69	.19	21.1	12.1
53	13.21	14.36	.86	6.5	13.4	.77	.57	.18	23.0	12.1
54	13.26	15.00	1.29	9.7	13.6	.77	.56	.18	24.0	12.8
55	12.15	14.33	1.78	14.6	16.2	.52	.42	.11	22.0	12.8
56	14.01	16.42	1.86	13.3	14.2	.62	.52	.17	27.5	13.2
57	14.31	16.50	1.62	11.3	13.7	.86	.68	.14	16.4	11.3
58	14.15	15.62	1.30	9.2	11.0	1.02	.80	.17	16.3	11.8
59	14.80	16.21	.94	6.3	12.8	.91	.67	.19	20.8	11.4
60	15.03	16.36	1.02	6.8	12.5	.76	.55	.17	22.4	12.1
61	14.66	16.45	1.26	8.6	14.1	.65	.40	.16	24.7	13.4
62	14.76	16.23	1.16	7.8	14.3	.60	.47	.16	27.5	13.3
63	14.86	16.18	1.03	7.0	---	.68	.50	.12	18.1	---
64	15.22	16.86	1.26	8.3	12.7	.84	.65	.16	19.5	11.6
65	15.29	17.08	1.34	8.8	14.3	.60	.36	.16	27.3	12.2
66	16.75	17.72	.77	4.6	13.8	.77	.53	.19	24.7	12.0
67	15.59	16.98	.94	6.0	13.8	.74	.57	.12	15.7	11.8
68	15.41	16.83	1.23	8.0	12.5	.72	.52	.23	31.6	13.2
69	17.50	18.93	1.08	6.2	13.4	.56	.41	.12	21.0	12.0
70	16.64	17.98	1.05	6.3	14.2	.40	.16	.17	43.1	11.8

* Generation 0 = 1896; Generation 45 = 1941 and Generation 46 = 1945, years are consecutive from 1896 to 1941 and 1945 to 1969. † Standard error of an observation.

variances, standard deviations, and coefficients of variation were calculated for all generations of each strain.

Average change per generation was calculated from the means predicted from regression of means on cumulative selection differential as the difference between the last generation of one segment and the last generation of the preceding segment divided by the number of generations in the segment.

Inbreeding coefficients were calculated for each strain using the relationship $F = 1/8N_m + 1/8N_F$ (Falconer, 1960) where N_m = effective number of males and N_F = effective number of females. From generations 0 to 9 and 29 to 70 the effective number of males and of females was equal to the number of ears saved. For generations 10 to 24, there were 6 females and 12 males, whereas from generations 25 to 28 there were 12 males and 12 females. From the F values for each generation a panmictic index ($P = 1 - F$) was calculated; P for the 70th generation was computed as the product of all the P values. F_{70} was then calculated as $1 - P_{70}$.

Chemical Analysis

Sampling and chemical analytical methods have been described by Hopkins (1898), Smith (1908), Hoener and De Turk (1938) and Leng (1962a). Since 1962, a slightly modified Kjeldahl-Gunning-Arnold method has been used for nitrogen determination with % protein = % N X 6.25. Before 1964, oil percentage was determined by 16-hour extraction of ground, whole-grain samples with Skellysolve F in a Butt apparatus. Since 1964, oil percentages have been determined by nuclear magnetic resonance (NMR) as outlined by Alexander et al. (1967).

Supplementary Experiments

In 1970, generations 65 to 70 of IHP, ILP, IHO, and ILO and generations 19 to 22 of RHP were tested in a 10-replicate randomized complete block trial at Urbana, Illinois. Plots consisted of 1 row, 13 plants long, with 76 cm between rows and 38 cm between plants within a row. Five to seven plants in each row were self-pollinated. Seed from five well-filled ears in each row were saved for chemical analysis. The experiment was repeated in 1971 with the addition of the latest generation to each experiment.

In a separate experiment during 1970-1972, a yield trial including the 70th generation of all 9 strains was grown in a 3-replicate randomized complete block design. Plots consisting of 3 rows 14.4 m long with 76 cm between rows were thinned to a stand of 44,500 plants/ha. The center row was harvested for yield.

RESULTS AND DISCUSSION

Direct Response

Total

Smith (1908) reported that selection was effective in increasing and decreasing oil and protein percent. Winter (1929) suggested that IHP and IHO showed no indication of approaching an upper limit, ILP had changed very little in the preceding 20 years, and ILO was approaching a physiological lower limit. Leng (1961) interpreted the data through 60 generations as indicating that the selection limit had not been reached in any of the strains. Examination of Fig. 1 and 2 and Tables 3 to 7 reveals that, with the possible exception of RHO, progress has continued in all strains in the 10 generations following Leng's analysis.

The magnitude of the response can be illustrated by considering change as percent of the original mean. For this purpose, the mean of generation 70 predicted from the regression of the mean on cumulative selection differential was used. Change, as percent of the original mean, was 115, 77, 241, and 86% for IHP, ILP, IHO, and ILO, respectively. Because the lowest possible value for ILP and ILO is zero, the maximum change for these strains is 100%. In terms of standard deviations of the original population, generation 70 means were 12, 8, 27, and 10 S. D. beyond the original mean in IHP, ILP, IHO, and ILO, respectively. These changes were achieved by evaluating approximately 6,000 ears in each of the 4 populations (Table 1). Because only 2.6 X 10^{-12} individuals in a normally distributed population are expected to exceed the mean by as many as 7 S. D., mild selection combined with frequent opportunity for recombination have been powerful tools in achieving progress with a minimal number of observations. If the same number of ears had been analyzed in the original population, the most extreme ear expected would have been only 3.8 S. D. from the mean.

The effectiveness of selection also can be measured by the number of generations required before ranges of divergently selected strains no longer

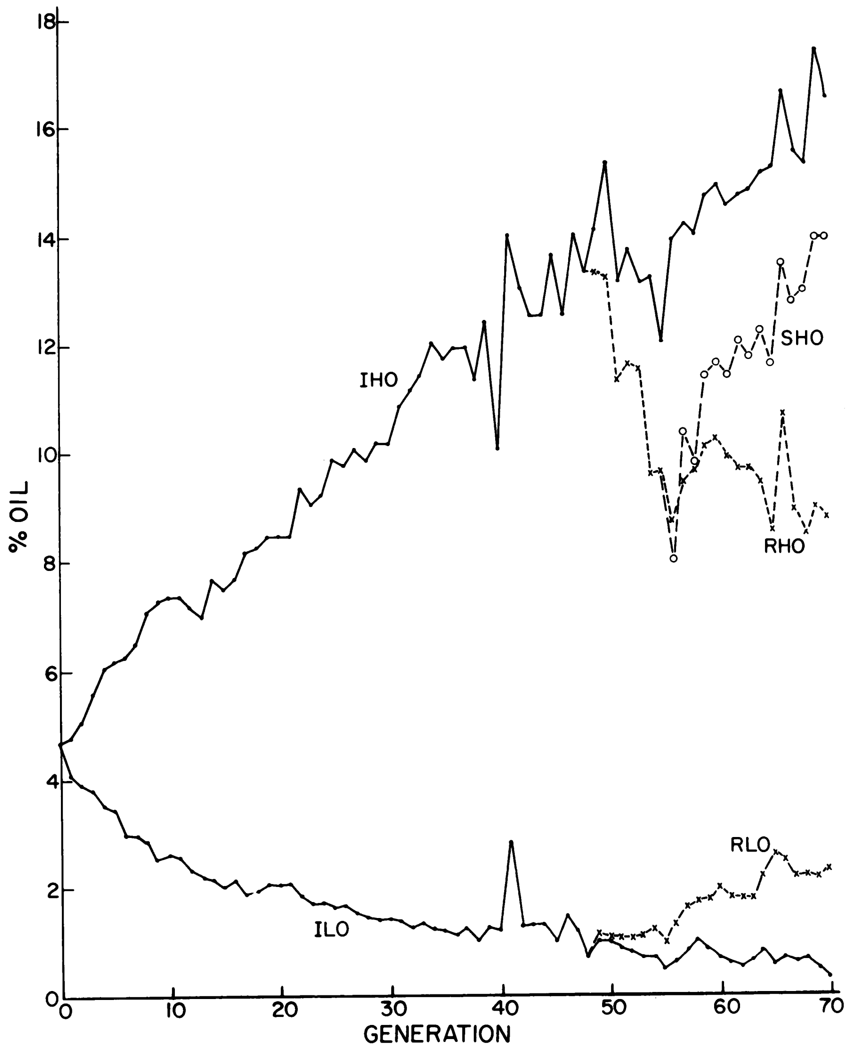

Figure 1. Mean percent oil for IHO, ILO, RHO, RLO, and SHO plotted against generations.

overlap. After only four generations the ranges of IHO and ILO no longer overlapped. However, 16 generations were required to separate the ranges of IHP and ILP. In the reverse selection strains, the number of generations required to separate the ranges was 11 for ILO and RLO, 14 for IHO and RHO, 13 for RHO and SHO, and 12 for IHP and RHP. The ranges of ILP and RLP still overlapped after 22 generations of selection.

On an individual ear basis, the highest and lowest protein percentages observed were 29.8 (generation 70, IHP) and 3.44 (generation 69, ILP). In

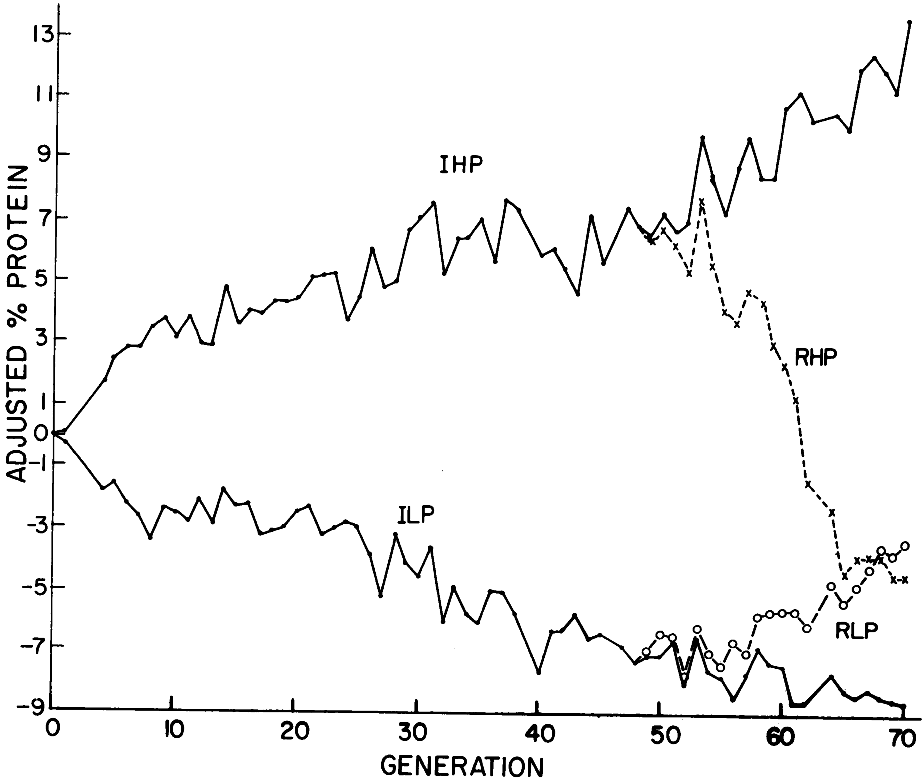

Figure 2. Mean adjusted percent protein for IHP, ILP, RHP, and RLP plotted against generations.

the oil strains the most extreme values recorded were 19.6% (generation 69, IHO) and .10% (generation 70, ILO). To our knowledge, these values encompass the total range of oil and protein percentages observed in maize.

Asymmetry

Leng (1962a) discussed the asymmetry of response of IHP compared to ILP and IHO compared to ILO. He suggested that asymmetry in the oil strains could be explained if change in percent oil in the germ and in percent germ in the kernel were considered separately. Subdividing the experiment into segments, comparing realized heritabilities, and evaluating the reverse selection data permit a more detailed analysis of the causes of asymmetrical response.

Differences in average change per generation in populations divergently selected from the same initial population indicate asymmetrical response. In the protein strains, comparisons of IHP with ILP for all four segments of the experiment and of IHP with RHP and ILP with RLP in the last segment

Table 4. Data from 70 generations of selection in Illinois High Protein and Illinois Low Protein.

	Illinois High Protein						Illinois Low Protein					
	Mean % Protein						Mean % Protein					
Generation*	All Ears	Select Ears	Adjusted†	S.E.‡	C.V.	% Oil	All Ears	Select Ears	Adjusted†	S.E.‡	C.V.	% Oil
0	10.9	12.5	0	1.05	9.6	4.69	10.9	9.0	0	1.05	9.6	4.69
1	11.0	12.5	.08	1.17	10.6	4.52	10.6	9.1	-0.26	1.33	12.5	4.35
2	11.0	13.1	---	1.23	11.2	---	10.5	8.4	---	1.33	12.6	---
3	11.6	13.7	---	1.27	10.9	---	9.5	8.1	---	1.01	10.6	---
4	12.6	14.7	1.71	1.02	8.1	4.75	9.1	7.7	-1.78	1.04	11.4	4.31
5	13.7	15.4	2.50	1.23	9.0	4.82	9.6	8.1	-1.53	1.11	11.5	4.30
6	12.9	14.1	2.84	1.10	8.5	4.85	7.9	7.1	-2.21	.73	9.3	4.15
7	13.5	15.2	2.83	1.44	10.7	4.83	8.0	7.0	-2.61	.84	10.5	4.08
8	15.0	16.7	3.45	1.35	9.0	5.07	8.2	7.3	-3.42	.86	10.5	4.17
9	14.7	16.1	3.74	1.24	8.5	5.04	8.6	7.4	-2.42	1.05	12.2	3.85
10	14.2	16.0	3.07	1.32	9.3	5.28	8.6	7.5	-2.53	.93	10.8	3.86
11	13.9	15.6	3.80	1.50	10.8	5.00	7.3	6.3	-2.77	.89	12.2	3.77
12	13.9	16.0	2.90	1.68	12.1	4.67	9.0	7.3	-2.08	1.25	14.0	3.69
13	13.3	15.0	2.90	1.47	11.1	5.01	7.5	6.4	-2.91	.95	12.7	3.70
14	14.9	16.5	4.77	1.44	9.7	4.79	8.2	7.3	-1.85	.89	10.8	3.60
15	13.8	16.3	3.63	1.80	13.1	4.70	7.9	6.7	-2.26	1.18	15.0	3.76
16	14.5	16.0	4.05	1.15	8.0	4.75	8.2	7.4	-2.20	.76	9.3	3.72
17	14.8	16.3	3.87	1.24	8.3	4.69	7.7	6.6	-3.25	.96	12.5	3.55
18	15.0	16.8	4.32	1.41	9.4	5.13	7.6	6.7	-3.07	.96	12.6	3.96
19	14.5	16.1	4.26	1.49	10.2	5.07	7.3	6.3	-3.01	.86	11.8	3.89
20	15.7	17.2	4.44	1.34	8.5	4.70	8.7	7.5	-2.54	1.01	11.6	3.99
21	14.4	16.7	5.09	1.85	12.8	4.75	7.1	6.3	-2.28	.69	9.7	3.62
22	15.5	16.9	5.15	1.38	8.9	4.66	7.1	6.4	-3.20	.71	10.0	3.96
23	14.7	16.6	5.28	1.55	10.5	4.83	6.4	5.9	-2.97	.54	8.3	3.72
24	14.0	16.1	3.73	1.79	12.8	---	7.5	6.5	-2.75	.91	12.1	3.90
25	16.7	18.4	4.53	1.82	10.9	4.60	9.1	7.7	-3.00	1.37	15.0	4.16
26	17.3	18.7	6.14	1.23	7.1	4.59	7.4	6.5	-3.78	.74	10.0	3.82
27	16.5	18.1	4.90	1.42	8.6	4.55	6.5	5.8	-5.15	.70	10.9	3.45
28	16.6	18.2	5.05	1.18	7.1	4.64	8.4	7.0	-3.17	1.16	13.8	3.58
29	18.3	19.9	6.68	1.34	7.3	4.71	7.5	6.5	-4.14	.83	11.1	3.74
30	18.2	19.5	7.18	1.09	6.0	4.86	6.5	5.7	-4.50	.67	10.3	3.90
31	18.4	19.9	7.59	1.15	6.2	4.97	7.2	6.3	-3.63	.85	11.7	3.70
32	18.3	20.2	5.31	1.65	9.1	5.06	6.8	5.8	-6.13	.94	13.8	3.90
33	17.7	19.9	6.46	1.84	10.4	4.94	6.3	5.7	-4.92	.69	10.9	3.64
34	20.1	21.3	6.55	1.04	5.2	5.17	7.8	6.4	-5.73	1.07	13.7	4.03
35	20.2	21.3	6.96	.93	4.6	4.62	7.1	6.3	-6.06	.69	9.6	4.17
36	19.4	21.4	5.66	1.58	8.1	5.11	8.7	7.3	-4.99	1.18	13.6	3.86
37	21.8	23.8	7.72	1.60	7.4	5.45	9.1	7.4	-4.98	1.30	14.4	3.83
38	23.8	24.9	7.36	1.20	5.0	4.52	10.7	8.6	-5.70	1.65	15.4	3.48
39	17.7	19.8	---	1.66	9.4	---	5.9	5.3	---	.52	8.9	---
40	21.6	23.8	5.93	1.67	7.7	4.45	8.0	7.1	-7.69	.73	9.1	2.28
41	18.6	20.7	6.14	1.84	9.9	6.70	6.1	5.3	-6.28	.65	10.5	4.47
42	17.6	20.8	5.54	2.53	14.4	6.48	5.8	5.3	-6.23	.44	7.6	5.07
43	15.6	19.5	4.55	2.77	17.7	5.52	5.4	4.9	-5.72	.39	7.2	3.55
44.	19.9	21.7	7.20	1.57	7.9	5.16	6.2	5.5	-6.53	.74	11.9	3.36
45	17.8	21.1	5.58	2.32	13.0	5.72	5.8	5.2	-6.39	.51	8.8	3.19
46	17.6	20.6	---	2.15	12.2	---	6.3	5.5	---	.72	11.4	---
47	20.1	22.0	7.51	1.69	8.4	5.15	5.8	5.1	-6.80	.61	10.6	3.13
48	19.2	21.2	6.86	1.42	7.4	4.48	5.1	4.6	-7.26	.42	8.2	2.65
49	19.1	21.7	6.58	2.13	11.2	5.20	5.5	4.8	-7.06	.54	7.8	2.99

(Continued)

50	19.3	20.7	7.28	1.42	7.4	5.84	4.9	4.4	-7.10	.40	8.2	3.00
51	18.9	21.7	6.70	2.29	12.2	4.93	5.6	4.8	-6.60	.78	14.0	3.08
52	20.3	22.6	7.04	1.88	9.3	5.52	5.2	4.6	-8.02	.52	9.9	3.07
53	22.5	24.1	9.76	1.09	4.8	4.30	6.3	5.2	-6.49	1.24	19.9	2.84
54	21.7	23.2	8.48	1.17	5.4	5.26	5.6	5.0	-7.61	.54	9.6	3.08
55	21.8	23.6	7.29	1.39	6.4	5.26	6.7	5.5	-7.82	1.25	18.7	2.56
56	22.4	23.7	8.68	.94	4.2	3.64	5.2	4.3	-8.49	.73	13.9	3.12
57	22.2	23.8	9.68	1.37	6.2	5.67	4.8	4.2	-7.66	.48	10.0	2.88
58	19.8	23.0	8.45	2.38	12.0	4.57	4.6	4.2	-6.81	.32	7.1	2.90
59	20.5	23.0	8.44	2.03	9.9	4.52	4.8	4.3	-7.31	.50	10.4	2.84
60	23.0	25.5	10.73	2.09	9.1	5.12	4.9	4.5	-7.40	.35	7.1	3.16
61	24.9	26.9	11.18	1.37	5.5	3.95	5.2	4.6	-8.57	.46	8.9	3.11
62	24.0	26.1	10.22	1.92	8.0	4.84	5.2	4.4	-8.62	.63	12.1	3.20
63	23.3	25.1	---	1.38	5.9	---	4.4	4.0	---	.35	8.0	2.93
64	22.5	25.2	10.37	2.18	9.7	4.67	4.5	4.1	-7.65	.35	7.8	3.23
65	23.1	24.4	9.88	.94	4.1	5.10	5.1	4.5	-8.18	.62	12.2	2.96
66	24.9	26.0	11.97	1.02	4.1	3.71	4.4	4.0	-8.45	.43	9.7	3.31
67	25.2	27.1	12.41	1.49	5.9	5.70	4.6	4.3	-8.21	.29	6.3	3.21
68	24.7	26.4	11.89	1.52	6.1	5.91	4.5	4.1	-8.39	.34	7.6	2.91
69	23.9	25.9	11.18	1.54	6.4	5.35	4.2	3.9	-8.48	.23	5.3	2.92
70	26.6	28.6	13.65	1.69	6.3	4.82	4.4	4.1	-8.57	.23	5.3	3.10

* Generation 0 = 1896; Generation 45 = 1941 and Generation 46 = 1945, years are consecutive from 1896-1941 and 1945-1969. † Observed mean % protein—average % protein in IHO and ILO. ‡ Standard error of an observation.

Table 5. Data from 22 generations of selection in Reverse High Protein and Reverse Low Protein.

Generation	Reverse High Protein						Reverse Low Protein					
	Mean % Protein						Mean % Protein					
	All Ears	Select Ears	Adjusted†	S.E.‡	C.V.	% Oil	All Ears	Select Ears	Adjusted†	S.E.‡	C.V.	% Oil
0*	19.2	16.6	6.86	1.42	7.4	4.48	5.1	5.9	-7.26	.42	8.2	2.65
1	18.2	13.2	5.64	3.03	16.6	---	5.5	6.2	-7.03	.48	8.6	---
2	18.8	16.5	6.79	1.40	7.4	---	5.6	6.3	-6.40	.41	7.3	---
3	18.4	14.1	6.26	2.77	15.1	---	5.6	6.7	-6.52	.67	11.8	---
4	18.7	15.9	5.42	1.93	10.3	---	5.4	6.0	-7.80	.45	8.2	---
5	20.6	18.4	7.82	1.45	7.1	---	6.5	7.8	-6.21	.80	12.2	---
6	18.8	17.1	5.64	1.17	6.2	4.48	6.2	7.3	-6.99	.64	10.3	2.94
7	18.6	16.8	4.13	1.36	7.3	5.62	7.1	8.2	-7.45	.71	10.1	2.54
8	17.4	15.3	3.69	1.43	8.2	3.36	7.2	8.6	-6.56	1.09	15.3	2.56
9	17.3	15.9	4.84	1.12	6.5	4.76	5.5	6.3	-6.97	.54	9.7	2.81
10	15.7	13.4	4.37	1.58	10.0	4.38	5.6	6.7	-5.73	.76	13.5	2.76
11	15.1	12.1	2.96	1.96	13.0	4.26	6.5	8.6	-5.57	1.54	23.5	2.73
12	14.7	12.5	2.39	1.82	12.4	5.13	6.8	8.1	-5.56	.91	13.5	3.38
13	15.1	12.7	1.34	1.69	11.2	4.14	8.1	9.6	-5.62	1.09	13.4	3.05
14	12.3	10.3	-1.50	1.54	12.5	4.19	7.7	9.3	-6.13	1.07	14.0	3.22
15	10.8	9.5	---	1.20	11.1	---	7.9	9.2	---	1.02	13.0	---
16	9.7	8.8	-2.42	.77	7.9	3.82	7.4	8.9	-4.73	.97	13.2	3.12
17	8.8	8.1	-4.44	.54	6.2	---	7.9	9.2	-5.31	.91	11.5	---
18	9.0	8.3	-3.87	.57	6.3	3.70	8.0	9.3	-4.84	1.00	12.4	3.35
19	9.1	8.3	-3.75	.66	7.3	---	8.6	10.0	-4.19	.91	10.5	---
20	9.1	8.3	-3.79	.56	6.2	4.22	9.3	10.9	-3.54	1.13	12.1	3.44
21	8.2	7.5	-4.53	.58	7.1	4.15	9.0	10.7	-3.67	1.14	12.6	3.68
22	8.5	7.7	-4.43	.66	7.8	4.26	9.6	11.6	-3.42	1.67	17.5	3.55

* Generation 48 of Illinois High Protein, 1947. † Observed mean % protein—average % protein in IHO and ILO. ‡Standard error of an observation.

Table 6. Data from 22 generations of selection in Reverse High Oil and Reverse Low Oil.

Generation	Reverse High Oil					Reverse Low Oil				
	Mean % Oil				% Protein	Mean % Oil				% Protein
	All Ears	Select Ears	S.E.†	C.V.		All Ears	Select Ears	S.E.†	C.V.	
0*	13.45	11.79	.92	6.9	12.5	.76	1.03	.16	20.5	12.2
1	13.45	12.08	.93	6.9	---	1.10	1.35	.18	16.2	---
2	13.32	11.06	1.48	11.1	---	1.03	1.29	.18	17.3	---
3	11.37	9.57	1.19	10.5	---	1.13	1.42	.23	20.3	---
4	11.67	10.18	1.02	8.7	---	1.13	1.42	.23	20.4	---
5	10.65	9.03	1.05	9.9	---	1.15	1.42	.24	21.3	---
6	9.64	8.18	1.09	11.3	12.8	1.27	1.66	.30	23.5	13.4
7	9.69	7.94	1.42	14.7	13.5	1.05	1.38	.24	22.4	12.6
8	8.80	6.99	1.35	15.3	14.1	1.36	1.70	.24	17.8	14.0
9	9.56	7.89	1.22	12.7	12.6	1.67	1.96	.20	12.3	10.4
10	9.74	7.89	1.30	13.4	10.6	1.77	2.17	.30	16.7	10.4
11	10.16	8.47	1.15	11.4	9.8	1.82	2.13	.23	12.9	10.3
12	10.31	8.64	1.16	11.2	11.5	2.02	2.48	.34	16.6	11.2
13	9.97	8.49	1.14	11.5	12.0	1.84	2.11	.20	11.1	11.5
14	9.78	8.28	1.10	11.3	11.8	1.82	2.20	.25	13.7	10.8
15	9.78	8.30	1.02	10.4	---	1.81	2.06	.17	9.6	---
16	9.51	8.13	1.03	10.8	11.3	2.26	2.52	.20	9.0	10.5
17	8.61	6.51	1.44	16.7	---	2.65	3.04	.33	12.3	---
18	10.80	8.79	1.28	11.8	---	2.58	2.94	.24	9.5	---
19	9.03	7.88	.80	8.9	---	2.23	2.53	.23	10.3	---
20	8.54	7.35	1.01	11.8	---	2.23	2.57	.28	12.6	---
21	9.07	7.61	1.00	11.1	10.1	2.20	2.52	.28	12.6	10.5
22	8.85	7.56	.93	10.5	11.1	2.38	2.90	.35	14.5	10.6

* Generation 48 of Illinois Low Oil and Illinois High Oil, 1947.

† Standard error of an observation.

Table 7. Data from 15 generations of selection in Switchback High Oil.

Generation	Mean % Oil		S.E.†	C.V.
	All Ears	Selected Ears		
0*	9.69	11.83	1.42	14.7
1	8.03	9.24	.90	11.2
2	10.40	12.30	1.31	12.6
3	9.85	11.67	1.16	11.8
4	11.48	13.13	1.29	11.3
5	11.71	13.17	1.04	8.8
6	11.47	13.33	1.38	12.0
7	12.15	13.52	1.04	8.6
8	11.84	13.19	1.03	8.7
9	12.32	13.33	.91	7.4
10	11.76	13.28	1.06	9.0
11	13.58	14.64	.82	6.1
12	12.83	14.23	.94	7.4
13	13.01	14.40	1.00	7.7
14	14.05	15.84	1.31	9.3
15	14.02	14.96	.66	4.7

* Generation 7 of Reverse High Oil, 1954.

† Standard error of an observation.

measure asymmetry. If differences in cumulative selection differential are responsible for asymmetry of response, then realized heritability values in the divergent populations should be similar even if average change per generation is different.

Response in IHP and ILP was similar in segment 1 (Table 8). In segment 2, change per generation was 2.5 times as high in IHP as in ILP but the realized heritabilities were not significantly different (Fig. 3, Table 8). Thus,

Table 8. Change in percent protein per generation and realized heritabilities (h^2) for the protein strains.

Segment	Change/generation	h^2	Change/generation	h^2
	IHP		ILP	
1	.31	.20 ± .05	.24	.18 ± .04
2	.15	.08 ± .02	.06	.05 ± .02
3	.09	.04 ± .01	.16	.17 ± .01
4	.27	.15 ± .01	.05	.09 ± .03
	RHP		RLP	
1		−.12 ± .07		−.10 ± .14
2	.64	.40 ± .03	.19	.15 ± .02

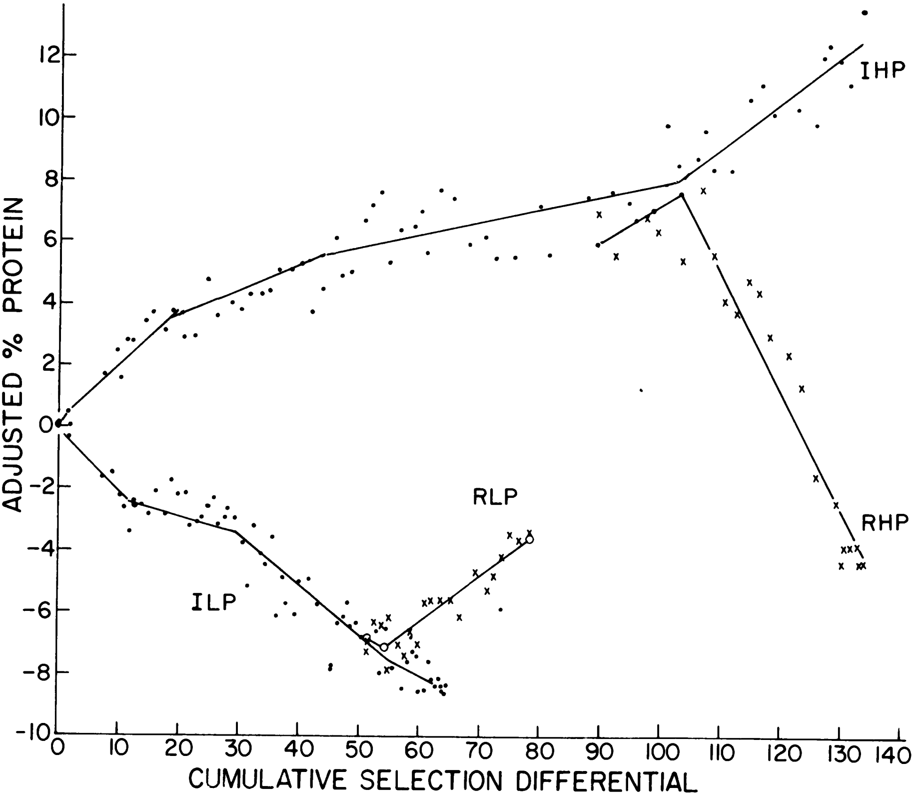

Figure 3. Mean adjusted percent protein for IHP, ILP, RHP, and RLP plotted against cumulative selection differential. Slopes of lines represent realized heritability values.

the asymmetry observed through generation 25 can be attributed to differences in selection differential. Reduced progress in segment 2 in both strains can be attributed in part to simultaneous selection for yield. In segment 3, change per generation and realized heritability were both higher in ILP than in IHP; however, in segment 4, response per generation and realized heritability increased drastically in IHP and decreased in ILP. The only difference between segments 3 and 4 was the application of N fertilizer in segment 4. Therefore, the slower response of IHP in segment 3 and the drastically increased response in segment 4 probably resulted from the lack of adequate soil N to permit maximum expression of high protein genotypes in segment 3. With the added soil N in segment 4, genotypes capable of producing higher percent protein in the presence of additional N could be identified and progress at a rate similar to that in segment 1 was achieved. The lower change per generation and realized heritability in ILP in segment 4 may have resulted from masking effects of higher N levels or a decrease in genetic variability.

Change per generation and realized heritability in RHP were approximately 2.5 times as high as in segment 4 of IHP (Table 8). Progress in RLP also was significantly higher than in the fourth segment of ILP. In RHP only 14 generations of reverse selection were required to reduce percent protein by an amount equal to that achieved by 48 generations of forward selection. If the confounding effects of selection for yield and inadequate N levels to support higher levels of protein are considered, the number of generations of forward selection required to reach the level observed after 48 generations would have been reduced. However, the realized heritability and change per generation in RHP were twice as high as the highest values in IHP, suggesting that reverse selection was at least twice as effective as forward selection.

The results from selection for percent oil are strikingly different from those observed for protein (Table 9, Fig. 1 and 4). In the first segment, change per generation and realized heritability in IHO and ILO were nearly the same. Change per generation in each of the last three segments was

Table 9. Change in percent oil per generation and realized heritabilities (h^2) for the oil strains.

Segment	Change/generation	h^2	Change/generation	h^2
	IHO		ILO	
1	.22	.34 ± .09	.21	.50 ± .05
2	.20	.31 ± .03	.06	.24 ± .03
3	.14	.14 ± .01	.02	.10 ± .02
4	.11	.08 ± .01	.02	.13 ± .04
	RHO		RLO	
1	.37	.22 ± .04	.09	.29 ± .05
2	.01	.01 ± .03	.07	.19 ± .04
	SHO			
1	.33	.21 ± .02		

markedly higher in IHO than ILO. However, the realized heritability values were not significantly different, indicating that the asymmetrical response resulted from differences in selection differential. The differences in cumulative selection differential are graphically illustrated in Fig. 4.

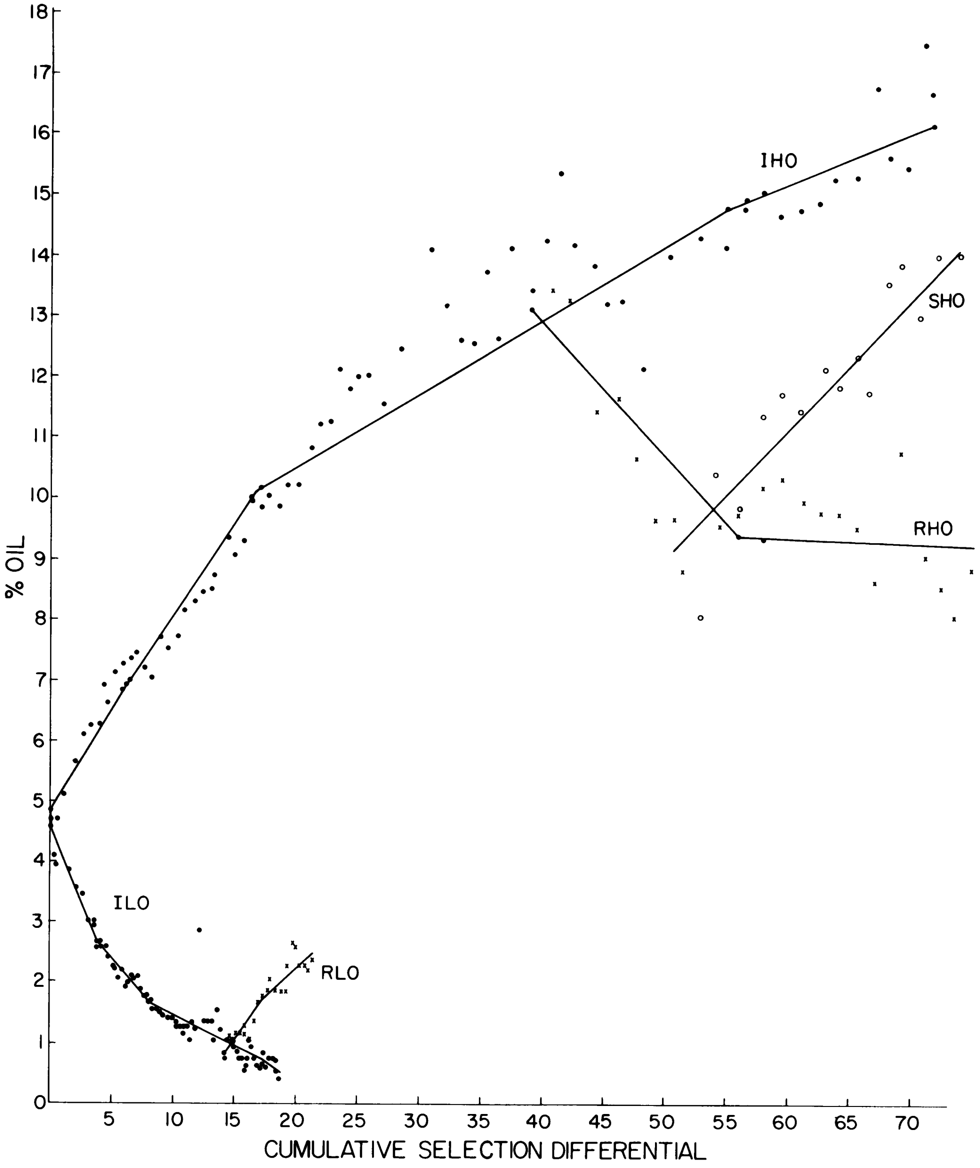

Figure 4. Mean percent oil for IHO, ILO, RHO, RLO, and SHO plotted against cumulative selection differential. Slopes of the lines represent realized heritability values.

In the first 10 generations of RHO, progress per generation was nearly twice as high as in any segment of IHO (Table 9). However, realized heritability was approximately midway between the highest and lowest values in IHO. Essentially no progress occurred during the last 12 generations of RHO. This was the only case of failure to achieve progress from selection observed in the entire experiment. SHO, initiated after 7 generations of selection in RHO, showed progress similar to the first 10 generations of RHO, indicating that despite the fact that most of the progress in RHO already had occurred, genetic variability still remained. If the rapid progress now occurring in SHO continues, the mean will eventually equal or exceed IHO. Change per generation in RLO was higher than in the corresponding segments of ILO. During the first 10 generations of RLO, realized heritability was nearly 3 times as high as in segment 3 of ILO. However, realized heritability in the last 12 generations of RLO was not significantly different from the last segment of ILO.

Estimates of additive genetic variance ($\sigma_A{}^2$) paralleled the realized heritability values except for IHO and the first segment of RHO (Table 10). In IHO, estimates of $\sigma_A{}^2$ changed little from segment 1 to segment 4. The decline in realized heritability in IHO was offset by an increase in phenotypic

Table 10. Means, estimates of phenotypic variance ($\sigma_P{}^2$) and additive genetic variance ($\sigma_A{}^2$) genetic coefficients of variation ($G_{C.V.}$), and coefficients of variation (C. V.) by segments for all strains selected for chemical composition.

Strain	Segment	Mean	σ^2_P	σ^2_P	$G_{c.v.}$	C.V.
					%	
IHP	1	12.7	1.4558	.5823	6.00	9.6
	2	14.6	2.3366	.4732	4.71	10.4
	3	18.9	2.8525	.2282	2.53	8.9
	4	23.2	2.5147	.7544	3.74	6.7
ILP	1	9.3	1.1033	.3972	6.78	11.1
	2	7.8	.9055	.1111	4.27	11.8
	3	6.7	.6748	.2294	7.15	10.9
	4	5.0	.3536	.0636	5.04	10.0
IHO	1	5.97	.2272	.1545	6.58	8.0
	2	8.22	.3193	.2215	5.72	6.8
	3	12.42	.4767	.1334	2.94	7.3
	4	15.54	1.2135	.1942	2.84	7.1
ILO	1	3.51	.1067	.1067	9.31	9.1
	2	2.10	.0747	.0417	9.72	12.8
	3	1.22	.0500	.0100	8.20	18.7
	4	.69	.0277	.0072	12.30	24.7
RHP	2	13.3	1.5395	1.2316	8.34	8.6
RLP	2	7.5	1.0643	.3193	7.53	13.2
RHO	1	11.0	1.2476	.5489	6.74	11.0
	2	9.5	1.2100	.0242	1.64	11.4
RLO	1	1.22	.0532	.0308	14.38	19.0
	2	2.15	.0696	.0264	7.56	12.1
SHO	1	11.8	1.2111	.5087	6.04	9.5

variance. In RHO, the phenotypic variance in the first segment was nearly the same as in segment 4 of IHO and realized heritability was nearly tripled, which resulted in a high estimate of σ_A^2. Changes in the genetic coefficient of variation paralleled those for realized heritability.

In all four cases of reverse selection, initial change per generation and realized heritability were higher than in continued forward selection. These results support the suggestion of Mather and Jinks (1971) that selection builds up blocks of genetic material which act as large effective factors. This concept is further supported by the rapid progress made in SHO. However, if realized heritability values from the reverse populations are compared with those from the segment of the forward strains preceding initiation of reverse selection, only RHP had a significantly higher realized heritability. The low realized heritability in segment 3 of IHP may have resulted from limiting levels of soil N.

Additive genetic variance and hence progress from selection are functions of gene frequency (Falconer, 1960). If, at a given generation, the frequency of genes favorable for progress in a forward direction is higher than that for unfavorable genes, the rate of continued forward progress will be slower than in preceding generations because additive genetic variance (maximum at intermediate gene frequencies) will decrease as further increases in favorable gene frequency occur. If selection is reversed, the frequency of genes favorable for progress in the reverse direction is lower than the unfavorable and hence progress from reverse selection proceeds at an increasing rate. Thus, the results from reverse selection may be as easily explained by gene frequency changes as by the concept of Mather and Jinks (1971).

Within-Strain Variation

Changes in within-strain variation were analyzed in an attempt to determine the effects of selection on genetic variation. Winter (1929) reported a detailed analysis of changes in means, coefficients of variation, and standard deviations for the first 28 generations of selection in IHP, ILP, IHO, and ILO. He concluded that variability measured by the standard deviation increased as the mean increased and coefficients of variation increased as the mean decreased.

The major changes in the coefficients of variation and phenotypic variances resulting from selection can be seen by comparing average values for the different segments of the experiment (Table 10). Values for each generation are given in Tables 3 to 7. In IHP the variance changed little from segment 1 to 4 but the coefficients of variation decreased because the mean increased. Variances in ILP decreased steadily from segment 1 to segment 4 with little change in coefficients of variation. In RHP, the variances and coefficients of variation for the first 15 generations were similar to those in IHP (Table 5). Variances in the last 7 generations were markedly lower and similar to those in segment 3 of ILP. The means for RHP in these generations were also similar to those in segment 3 of ILP. Mean variances of segments in IHO and ILO were almost linearly related to the means with the variance of segment 4 of IHO the highest and segment 4 of ILO the lowest.

A more detailed evaluation of the relationship of changes in means to changes in variability was made by evaluating correlations of means with variances and coefficients of variation (Table 11). In all strains except IHP, RHO, and SHO, the correlations between means and variances were positive and significant. Since IHP, RHO, and SHO are the only populations in which nearly all the means are above 10%, this result is in accord with the theory that means and variances are correlated if percentage data are in the range of 0 to 10%. This observation was confirmed by correlating the means and variances in the first 26 and the last 44 generations of IHO separately. In the first 26 generations, means ranged from 4.6 to 10% and in the last 44 from 10.0 to 17.5%. There was little correlation between means and variances (r = .151) above 10% oil and a highly significant correlation (r = .633) below 10% oil. As expected, significant negative correlations between means and coefficients of variation were obtained in the three populations (IHP, RHO, and SHO; Table 11) in which means and variances were not significantly correlated. The means and coefficients of variation also were negatively correlated in ILO and RLO. In these populations, the means continued to decrease after the variances reached a minimum. Thus, the means and variances in ILO were positively correlated because of the reduction in means after reduction in variances ceased. In ILP the means and coefficients of variation were positively correlated as were the means and variances, indicating that the variances decreased relatively more rapidly with selection than the means. Because of these various types of association, it is difficult to use changes in means, phenotypic variances, and coefficients of variation to assess the presence or lack of genetic variability.

Results from reverse selection demonstrated the existence of genetic variability after 49 generations of selection in all 4 populations (Leng, 1962b). Analysis of half-sib families by Dudley and Lambert (1969) re-

Table 11. Correlations of means with variances and coefficients of variation in the Illinois long-term chemical selection experiment.

	Correlation of mean with		
Population	Variance	C.V.	N†
IHP	.072	-.582*	66
ILP	.818*	.480*	66
IHO	.611*	-.048	71
ILO	.597*	-.735*	71
RHP	.521*	.250	23
RLP	.687*	.378	22
RHO	-.018	-.627*	23
RLO	.520*	-.797*	23
SHO	-.336	-.777*	16

* Significance at the .01 probability level. † Number of values correlated.

Table 12. Mean (1970-71) percent protein of latest generations of protein populations and percent oil of oil populations grown in the same years.

Generation	IHP	ILP	RHP	IHO	ILO†
65	24.9	5.47		15.9	1.02
66	24.7	5.66		16.2	.83
67 (19)‡	24.8	5.00	9.21	16.2	.81
68 (20)	24.6	5.10	9.05	16.8	.74
69 (21)	25.5	4.84	8.68	16.9	.66
70 (22)	25.5	4.60	8.45	16.7	.76
$S_{\bar{X}}$	.40	.28	.12	.20	.05
b §	.18*	-.19*	-.26**	.19*	-.05**

* and ** = significant sum of squares due to linear regression when tested against the error mean square at the .05 and .01 probability levels, respectively. † Data from 1970 only.
‡ Generation of RHP. § b = regression of $\bar{X}$ on generation.

vealed significant genetic variability after 65 generations of selection. Significant realized heritability values in the last segment of all strains except RHO (Tables 8 and 9) provided additional evidence that genetic variation had not been exhausted.

Significant regressions of means on generation number in the supplementary experiments which compared the latest generations in the same year (Table 12) provide further evidence that progress is continuing in IHO, ILO, IHP, ILP, and RHP. Examination of the means reveals that progress was not continuous but tended to occur in particular generations. In IHO significant mean differences were found between generations 65 and 66 as well as between 67 and 68, in ILO between generations 65 and 66, in IHP between generations 68 and 69 and in ILP between 66 and 67 and from 68 to 70. The b values in Table 12 (regression of mean on generation) estimate average change per generation for the last six generations. Compared to the last segment of the experiment (Tables 8 and 9) change in the last six generations was greater in IHO and ILP and less in ILO, IHP, and RHP.

Inbreeding Coefficients

Leng (1962a) suggested, based on yields reported by Woodworth, Leng, and Jugenheimer (1952) that the level of inbreeding in IHO, ILO, IHP, and ILP after 50 generations was in the range of 75 to 87.5%. Based on our calculations, inbreeding coefficients of the 9 strains at the end of 70 generations ranged from .82 to .83 (Table 13). Inbreeding within these strains, at present, is similar to the level of inbreeding within an S_2 or S_3 line.

Effects of the selection procedure on increase in level of inbreeding per generation are directly related to the number of parents saved. Thus, maximum inbreeding per generation occurred in generations 10 to 24 in which 6 females and 12 males were saved each generation. Ironically, that breeding procedure was adopted in an attempt to reduce the amount of inbreeding.

Table 13. **Inbreeding coefficients of 9 chemical strains after 70 generations of selection.**

Population	F*
IHP	.8182
ILP	8278
IHO	.8208
ILO	.8263
RHP	.8220
RLP	.8315
RHO	.8246
RLO	.8300
SHO	.8246

* Calculated on basis given in text.

Falconer (1960) presented an equation

$$h_t^2 = [h_o^2\,(1-F_t)]\,/(1-h_o^2F_t)$$

where h_t^2 = heritability in generation t, h_o^2 = heritability in the noninbred population, and F_t = coefficient of inbreeding in generation t for calculation of heritability within a line given the heritability in the original line. This equation is based on the assumption that all gene effects are additive. Using the realized heritabilities from the first segment of the experiment as h_o^2, predicted heritabilities were calculated for generation 70 (Table 14). For IHO and ILO the realized heritabilities for segment 4 were close to those predicted on the basis of adjusting for inbreeding. However, the h_t^2 values for IHP and ILP were considerably lower than the realized heritability values. Falconer points out that actual heritabilities may be higher than predicted if dominance or natural selection is important. In this experiment, selection was always exerted for the characters for which heritability was measured. Thus, one would expect realized heritability to be lower than predicted on the basis of inbreeding alone. However, inbreeding alone could account for the reductions in heritability noted in all four populations. The better agreement between the predicted and realized heritabilities in the oil strains may be a result of 1) less environmental influence on percent oil or 2) more completely additive genetic control of percent oil than of percent protein.

Table 14. **Predicted heritabilities (h_t^2) for generation 70 based on reduction in heritability resulting from inbreeding and realized heritability in segment 4.**

Strain	h_t^2	Realized h^2 Segment 4
IHP	.042	.149
ILP	.037	.090
IHO	.084	.076
ILO	.143	.126

Correlated Response

Oil and Protein

The phenotypic correlation between percent oil and percent protein in the original population was only .019. Unless the genotypic correlation was vastly different from the phenotypic, little change in percent oil would be expected from selection for percent protein or in percent protein from selection for percent oil. However, Smith (1908) and Hopkins, Smith, and East (1903) reported that divergent selection for percent protein had produced a divergence in percent oil. Smith (1908) noted a trend for percent protein to diverge in the oil populations. Dudley and Lambert (1969) found that in the 66th generation grown in 1966 and 1967, protein content in both IHO and ILO was higher than in generation 0. ILP was lower and IHP slightly higher in percent oil than generation 0.

The mean percent oil in the first 10 generations of IHP (4.89) was not significantly different from that of the last 10 generations (4.92). By contrast, the mean percent oil of the first 10 generations of ILP (4.15) was higher than the mean for the last 10 generations (3.21) and lower than the mean for the first 10 generations of IHP. The means for percent protein in the last 10 generations of both IHO (13.7) and ILO (12.4) were higher than in the first 10 generations (IHO = 11.4, ILO = 10.4). If a correlated response existed in IHO and ILO it was overshadowed by environmental changes which caused a general increase in protein level.

Significant positive phenotypic correlations between percent oil and percent protein were obtained in ILP, RLP, and IHO (Table 15). Significant negative correlations were obtained in ILO and RLO. The situation in the oil strains is anomalous. If a true correlated response had occurred, the

Table 15. Simple and partial correlations over generations used to measure correlated response of percent oil and percent protein.

Characters Correlated	Population	Simple Correlation	Partial Correlation†
Adjusted % protein vs % oil:	IHP	.032	
	ILP	.715**	
	RHP	.458	
	RLP	.842**	
% protein vs % oil:	IHO	.601**	.298*
	ILO	-.512**	.206
	RHO	.056	-.034
	RLO	-.710**	-.362
% oil	IHP vs ILP	.148	
% oil	RHP vs RLP	-.184	
% protein	IHO vs ILO	.756**	
% protein	RHO vs RLO	.825**	

* and ** Significant at the .05 and .01 probability level, respectively. † Correlation between protein and oil within the population holding percent protein constant in ILO for IHO, in IHO for ILO, in RHO for RLO and in RLO for RHO.

correlations in IHO and ILO would have had the same sign. Correlations of percent protein in IHO with percent protein in ILO and of percent protein in RHO with percent protein in RLO were positive and highly significant, suggesting that the variation in protein content among years was primarily environmental in nature. The partial correlation of percent protein vs percent oil in one oil strain, holding percent protein in the other constant (Table 15), was significant only for IHO at the .05 probability level. Thus, selection for percent oil had little influence on percent protein.

Since percent oil and percent protein in IHP were not significantly correlated and the difference in mean percent oil between the first and last generations was small, selection for high protein had little effect on percent oil. The reduced oil percent and highly significant correlation of percent oil and protein in ILP and RLP indicate that selection for low protein has reduced percent oil. The small nonsignificant correlations of percent oil in IHP with percent oil in ILP and in RHP with RLP suggest that variation in percent oil in the pairs of strains was not the result of environmental effects common to particular years.

Although reduced percent oil in ILP (Table 4) could be due to chance selection of low oil ears, most of the reduction occurred in the first and third segments of the experiment when realized heritability for percent protein was highest (Table 8). The increase in percent oil in RLP also occurred in the segment in which realized heritability was similar to that of the third segment of ILP. The change in percent protein during the third segment of of the experiment spanned approximately the same range in percent protein as in the duration of selection in RLP. These results suggest that reducing percent protein from 9 to 5% may have caused a reduction in percent oil.

Yield

The first data on yield in IHP, ILP, IHO, and ILO were reported by Smith (1908) for the years 1903 to 1906. As an average of the 4 years, ILP and ILO had nearly identical yields (Table 16) whereas yields of IHO and IHP were lower with IHP having the lowest yields. Yields were also obtained from 1918 to 1927 with 'Reid's Yellow Dent' (RYD) included as a check each year. Regression of mean yields of IHO, ILO, IHP, and ILP on yield of RYD accounted for 84.3, 83.9, 84.6, and 67.7%, respectively, of the variation in yield of the selected strains. Thus, yields changed little from 1912 to 1927. However, the mean yields from 1912 to 1927 of IHP, ILP, IHO, and ILO were 85.8, 85.7, 85.3, and 79.6%, respectively, of the 1903-1906 means suggesting that yield of ILO had declined relative to the other strains. The ranking of the strains for yield was unchanged.

The only critical comparisons of changes in yield as a result of selection other than the 1912-1927 data are between 1949 and 1970-71. The double cross, U. S. 13, had similar yields in both 1949 and 1971 (Table 16). Because the 1949 data came from the generation when reverse selection was

Table 16. Yield (bu/A) by years for the forward and reverse selected strains.

Year	IHP	ILP	IHO	ILO	Check
1903	27.3	37.7	32.7	41.3	---
1904	32.1	55.5	41.9	40.5	---
1905	56.6	60.7	58.4	58.1	---
1906	65.1	73.2	66.3	83.2	---
Mean	45.3	56.8	49.8	55.8	
1912	26.3	32.6	31.5	31.8	44.2*
1913	27.0	34.1	28.3	23.3	44.0*
1914	38.7	47.5	39.8	46.3	61.1*
1915	43.0	58.0	46.8	48.3	53.1*
1916	16.9	32.6	20.0	19.1	28.8*
1917	48.9	56.6	56.0	51.4	63.6*
1918	38.8	47.8	46.6	58.2	61.2*
1919	50.2	55.4	45.3	58.0	62.4
1920	55.8	70.1	63.2	65.0	76.4*
1921	39.8	57.8	47.7	48.3	62.5*
1922	46.3	52.5	51.6	56.7	69.7*
1923	48.5	52.6	52.6	51.3	65.7*
1924	37.8	38.2	41.4	39.0	52.5*
1925	30.5	47.5	27.5	32.4	46.1*
1926	33.5	44.8	36.1	37.1	59.5*
1927	40.7	51.2	45.6	44.4	59.7*
Mean	38.9	48.7	42.5	44.4	56.9*
1936	42.4	46.0	31.8	63.6	77.2
1941	58.0	70.5	53.2	71.6	---
1949	51.0	56.0	55.0	56.0	106.0**
1970	32.0	55.4	45.3	27.3	---
1971	33.1	54.6	38.9	30.4	112.4**
Mean (1970-71)	32.6	55.0	42.1	28.9	---
	RHP	RLP	RHO	RLO	
1970	55	71	45	58	
1971	52.6	77.7	37.3	48.7	
Mean	54.0	74.5	41.0	53.5	

* Station Reid. ** U. S. 13.

initiated, effects of continued forward selection and reverse selection on yield can be evaluated. Continued forward selection in IHP from 1949 to 1970 resulted in a 36% reduction in yield as percent protein changed from 19.3 to 26.6. In contrast RHP went from 19.3 to 8.5% protein with little change in yield. Forward selection in ILP had little effect on yield. In RLP a 33% increase in yield occurred as percent protein increased from 4.9 to 9.6%. As mean oil percent in IHO increased from 15.4 to 16.6 yield declined by 23%. However, a similar decline in yield occurred in RHO where oil content was reduced to 8.8%. In ILO, yield decreased nearly 50% as oil content declined from 1.01 to 0.4%. Selection in RLO had little effect on yield even though oil content increased to 2.4%.

Since the levels of inbreeding of all the strains tested in 1970-71 were similar (Table 13) it is apparent that extreme alteration in chemical composition of the grain affects yield independently of levels of inbreeding. An increase in protein from 19 to 27% had a drastic effect on yield, yet decreasing it from 19 to 8.5% had little effect. Comparison of yield of ILP and RHP suggests that reducing percent protein from 8.5 to 4.9 had little effect. However, the increased yield of RLP over ILP suggests that in ILP, reduction in protein percent affected yield. Thus, for a range in protein from 4.9 to 9.0% the results were contradictory. In ILO, reduction of percent oil from 1.01 to 0.4% had a drastic effect on yield but increasing it to 2.4 in RLO had little effect. The results from IHO and RHO are again contradictory. Increasing percent oil from 15.4 to 16.6% and decreasing it to 8.8% both resulted in a 25% reduction in yield.

Kernel Weight

Data presented by Hopkins (1899), unpublished data from 1934, and data from 1962 to 1968 (Table 17) show that all four strains have suffered reductions in kernel weight. However, the largest reductions occurred in IHP and IHO. Even in 1899, Hopkins noted that kernel weight was greater in ILO than in IHO. The general reduction in kernel weight in all strains can be attributed to inbreeding depression. The differential reduction among strains can be attributed to the effects of selection. If the data from 1934 are typical, changes in kernel weight occurred in different segments of the experiment in the different strains. In ILP most of the reduction occurred after 1934, in IHO and ILO most reduction occurred prior to 1934, and in IHP reduction occurred in both phases of the experiment.

Reverse selection increased kernel weight in RHP, RLP, and RHO (Table 17). However, RLO showed a decrease in kernel weight compared with ILO. Because the reverse strains have approximately the same level of inbreeding as the regular strains, part of the decreased kernel weight in the regular strains must be attributed to response to changes in chemical compo-

Table 17. **One hundred kernel weights of nine strains at different stages in the chemical experiment.**

Strain	Year 1899*	1934	1962 to 1968	1972
	g			
IHP	37.2	22.2	17.6	13.7
ILP	33.7	31.4	25.0	20.3
IHO	34.5	20.2	20.2	15.0
ILO	42.0	34.4	34.6	34.3
RHP			22.4	18.3
RLP			34.1	29.7
RHO			24.0	19.0
RLO			30.3	27.7
SHO			21.0	16.0

* From Hopkins (1899).

sition. RLP from 1962 to 1968 had approximately the same percent protein as ILP in 1899 and kernel weights were nearly the same. However, RHP had nearly the same percent protein in 1962-1968 as ILP in 1899 and kernel weight was drastically less. Thus, changes in kernel weight in IHP have been greater than can be accounted for by changes in percent protein.

Most of the variation in yield among the protein strains can be accounted for by differences in kernel weight if the average 100 kernel weights from 1962 to 1968 are compared with the 1970-71 yields. Kernels of IHP are lighter than those of ILP suggesting that selection for high percent protein reduced kernel weight. However, kernels of RLP were heavier than those of ILP. In addition, despite similar levels of protein, kernels of RLP were heavier than those of RHP, again suggesting that selection in IHP has reduced kernel weight more than can be accounted for by changes in percent protein alone. The results from RLP suggest that extremely low percent protein also has resulted in reduced kernel weight.

Except for ILO, differences in yield among the oil strains and between the oil and protein strains are associated with kernel weight. The yield of ILO in 1970 was much lower than expected based on kernel weight.

Ear Length and Kernel Row Number

Data on ear length and row number are available only from 1905, 1906, 1946, and 1961 (Table 18). Ear length decreased in IHP, ILP, IHO, and ILO from 1905-06 to 1961 by from 1.5 to 3.5 cm (0.6 to 1.4 inches). In IHO and ILO, most of the reduction in ear length occurred prior to 1946. Number of kernel rows changed little from 1905-06 to 1961. However, the populations differed in row number with IHO having the most rows and ILO the least. This difference was established at least as early as 1905, as noted by Davenport and Reitz (1907).

Maturity and Plant Height

After 48 generations of selection, IHO was the earliest and shortest of the 4 original strains; IHP and ILP were intermediate in maturity and height,

Table 18. Length of ear and number of kernel rows in the forward selected strains at different stages of the selection experiment.

Year	IHP	ILP	IHO	ILO
		Ear length, cm		
1905-6	19	21	18	20
1946	17	19	16	18
1961	16	18	17	18
		Row number		
1905-6	13.7	14.4	15.2	13.0
1946	13.4	13.8	14.9	14.0
1961	13.0	14.4	15.6	12.0

Table 19. Maturity and plant height data (expressed as percent of U.S. 13) in generations 48 and 70.

	Maturity		Plant height	
	Gen. 48	Gen. 70	Gen. 48	Gen. 70
IHP	106	105	77	70
RHP		105		83
ILP	101	108	85	80
RLP		106		91
IHO	90	96	67	64
RHO		99		65
SHO		95		66
ILO	112	115	98	82
RLO		116		80
U.S. 13	68*	81*	268(cm)	223(cm)

* Maturity in Gen. 48 measured as days from planting to half-silk, in Gen. 70 as days to full silk.

whereas ILO was the latest and tallest strain (Woodworth et al., 1952). Data from the 70th generation (Table 19) show that this ranking for maturity has not changed in the last 22 generations. However, ILP plants were similar in height to ILO plants.

Reverse selection had little effect on maturity (Table 19) in any of the strains and little effect on height in RHO, SHO, or RLO. However, both RLP and RHP were taller than the strains from which they originated. These differences resulted from approximately equal increases in height of RHP and RLP and a reduction in height of IHP and ILP. We offer no explanation for these shifts. Correlation of height with protein percentage should have resulted in change in opposite directions in RLP and RHP. Both RHP and RLP now have protein percentages near the mean of the original population. If there is an optimum protein percentage for maximum plant height, then selection toward the optimum from either direction could result in increased height. The reduction in height of IHP, ILP, ILO, and RLO could be the result of inbreeding depression. However, levels of inbreeding in these strains are similar to those in IHO, RHO, and SHO which showed no reduction.

Components of Percent Oil

Percent oil in the kernel is a function of percent germ and percent oil in the germ since very little oil is present in the endosperm (Hopkins et al., 1903; Curtis, Leng, and Hageman, 1968). Percent germ is determined by weight of germ and weight of the kernel. By plotting all the available data (Table 20) on percent germ, percent oil in the germ, weight per 100 kernels, and weight per 100 germs against percent oil, a clear picture of the effects of selection for percent oil on its components emerges (Fig. 5).

Over the entire range of values, percent germ increased linearly at a constant rate as percent oil increased. Selection for high oil increased percent oil at a rate which paralleled the increase in percent germ. The increase in percent germ accompanying selection for high oil resulted first from an

Table 20. Percent oil, percent germ, percent oil in the germ, weight/100 kernels, and weight/100 germs for different generations in Illinois High Oil, Illinois Low Oil, Reverse High Oil, and Reverse Low Oil.*

Population	Year	% Oil Kernel	% Oil Germ	% Germ	g/100 Kernels	g/100 Germs
IHO	1964	16.4	56.2	29.0	17.4	5.06
IHO	1960	14.6	49.5	28.3		
IHO	1949	14.4	50.8	25.1		
IHO	1945	13.0	---	22.3		
IHO	1936	11.6	44.4	19.8	21.8	4.06
RHO	1960	10.0	46.3	21.0		
RHO	1964	9.1	49.0	18.3	18.1	3.33
IHO	1902	7.0	41.8	13.8		
IHO	1898	6.3	---	13.0	30.5	3.9
ILO	1898	3.4	---	8.7	35.5	3.1
ILO	1902	2.5	24.8	7.7		
RLO	1964	2.3	25.2	7.9	28.7	2.28
RLO	1960	1.8	24.4	7.4		
ILO	1945	1.4	---	7.0		
ILO	1936	1.2	11.4	7.9	31.0	2.30
ILO	1949	1.1	11.6	6.4		
ILO	1964	0.8	10.3	5.5	29.8	1.63
ILO	1960	0.6	8.5	7.5		

1902 data from Hopkins (1903); 1945 data from Schneider, Earley, and De Turk (1952); 1964 data from Curtis et al. (1968); 1898 data from Hopkins (1899); 1936, 1949, and 1960 data previously unpublished.

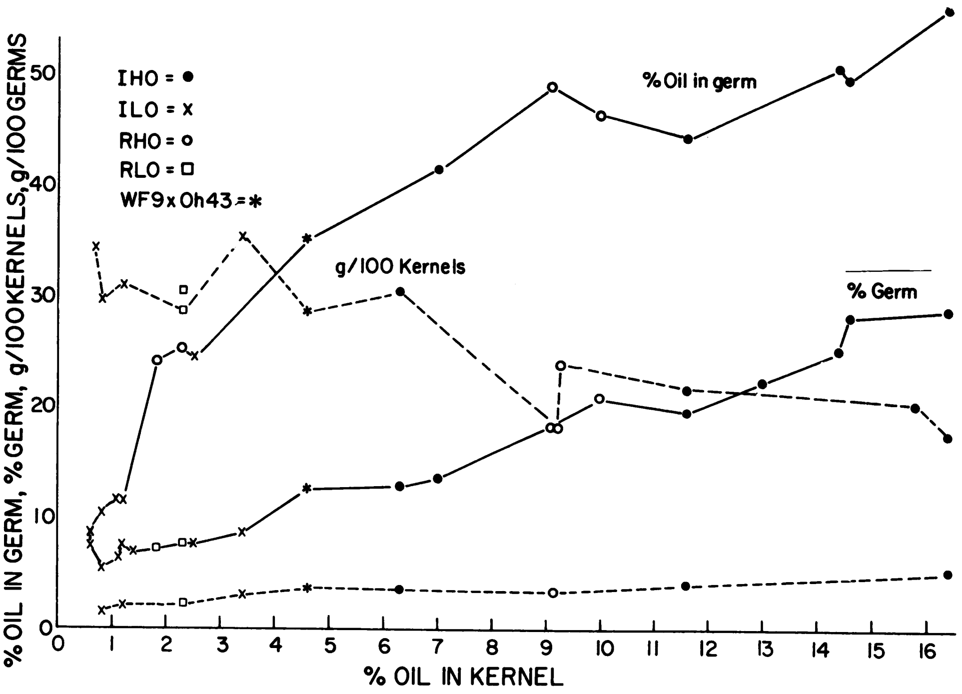

Figure 5. Graphical relationships of percent oil in the kernel with percent oil in the germ, percent germ, g/100 kernels, and g/100 germs. Data taken from Table 20.

overall reduction in kernel size which began after percent oil reached 6.3. Between 6.3 and 9.1% oil, weight of germ remained constant. Above 9.1%, weight of germ increased and the rate of decrease in kernel weights was reduced. Increased oil percent occurred in three stages. Initially, up to approximately 7% oil, the increase resulted primarily from an increase in percent oil in the germ. From 7 to 9.1%, percent oil in the germ continued to increase but percent germ also increased as a result of an overall decrease in kernel weight. Above 9.1%, percent oil in the germ, percent germ, and weight of germ increased while kernel weight continued to decrease.

Selection for low oil had little effect on kernel size. Most of the reduction in percent germ resulted from a decrease in weight of germ. The primary effect of selection for low oil was reduction in percent oil in the germ.

To the extent that kernel weight affects yield, the point at which increased percent oil causes a significant reduction in kernel weight becomes critical. From the data in Fig. 5, this value appears to be between 6.2 and 9.1% oil. Unpublished data from our laboratory, comparing percent oil and kernel weight for nine cycles of selection for high oil in each of two synthettics over a range from 5.2 to 9.8% oil and a period of 3 years show a negative linear relationship between kernel weight and percent oil above 7.0% oil. Significant decreases in yield occurred above 8.0% oil in both synthetics.

The negative relationship between kernel weight and percent oil reported here differs from the results reported by Alexander and Seif (1963) who found nonsignificant correlations between kernel weight and percent oil in two synthetics. However, the ranges in their synthetics were from 4.2 to 9.5 and 3.1 to 6.3% oil. Thus, most of their material fell in the range in which selection for increased oil had little effect on kernel weight.

Components of Percent Protein

Unlike oil, protein is a major component of both the germ and endosperm. A comparison of IHP and ILP in 1947 (Table 21) shows that the lower percent protein in ILP resulted from a major reduction in percent protein in the endosperm, a reduction in percent germ, and a small reduction in percent protein in the germ. The reduction in percent germ was due entirely to higher endosperm weight in ILP. Weight per 100 germs was not different.

Comparisons of IHP in 1902, 1945, and 1947 suggest that the differences in whole kernel protein between 14.4 and 20.4% resulted almost entirely from differences in percent protein in the endosperm (Table 21). The differences in percent germ between 6.6% (ILP, 1945 and 1947) and 17.0% protein (IHP, 1945) were as large as for 6.6 and 20.4% (IHP, 1947). Differences in percent protein in the germ between 6.6 and 14.4% whole kernel protein were as large as for 20.4 and 6.6% whole kernel protein. Thus, percent germ may have been one of the first components altered as percent protein diverged, followed by increased percent protein in the endosperm.

Table 21. Percent germ; weight of germs, kernels, and endosperms; and percent protein in kernel, endosperm, and germ in Illinois High Protein and Illinois Low Protein.

	IHP			ILP		
	1902*	1945*	1947†	1902	1945	1947
% germ	11.4	12.7	12.7	9.3	8.4	8.9
g/100 germs			2.45			2.65
g/100 kernels			19.2			29.6
g/100 endosperms			14.7			22.9
% protein: Kernel	14.4	17.0	20.4	6.7	6.6	6.6
Germ	21.2	20.4	22.0	18.0	17.9	17.9
Endosperm	13.8	17.9	21.4	5.7	5.6	5.3

* From Schneider et al. (1952). † From Watson (1949).

Other Long-Term Selection Experiments

Falconer (1960) summarized the results of long-term two-way selection experiments in *Drosophila* and mice. In general, response continued for 20 to 30 generations and the total range was 10 to 20 times the original phenotypic standard deviation (σ_P) or 15 to 30 times the square root of the additive variance (σ_A) in the original population. A more recent report (Wilson et al., 1971) covering 84 generations of selection for high body weight in the mouse showed progress through approximately 35 generations. Total response (one direction only) was approximately seven times the original phenotypic standard deviation.

Jones, Frankham, and Barker (1968) in contrast with most other long-term selection experiments found progress still continuing at the end of 50 generations of selection for bristle number in *Drosophila.* Response varied with population size and selection intensity.

Falconer (1971) reported two-way selection for litter size in mice. In his population, plateaus were reached after 20 generations in the low line and 35 in the high line. Total response was only 1.8 σ_P or 3.8 σ_A.

In nearly all cases of long-term selection reported to date, the populations studied have plateaued, response has been asymmetrical, and reverse selection effective.

The results from long-term selection for percent oil and percent protein in maize are similar to the results in laboratory animals in that selection was effective in both high and low directions and reverse selection produced more rapid changes than continued forward selection. A unique feature with maize was the continued response in both directions for the entire duration of the experiment. In addition, observed asymmetry of response can be accounted for by either differences in selection differential, changes in the selection procedure, or changes in cultural practices.

The total range (i.e., the difference between high and low lines at the last generation reported) is expressed in Table 22 in terms of the original

Table 22. Total range as percent of mean and multiple of σ_P or σ_A.

Strains Compared	Total Range		
	% Mean	/σ_P	/σ_A
IHP vs ILP	192	20	21
IHP vs RHP	100	12	
ILP vs RLP	117	11	
IHO vs ILO	332	37	43
IHO vs RHO	52	7	
ILO vs RLO	233	12	

mean, σ_P and σ_A. For percent protein the value of 20 σ_P is similar to that reported by Falconer (1960) for abdominal bristles in *Drosophila.* However, for percent oil the range of 37 σ_P and 43 σ_A are larger than any values found in the literature as is the value of 332% of the original mean. The total ranges of reverse selection in RHP and RLP of 12 and 11 σ_P, respectively, are quite similar despite the fact that realized heritability in RHP was greater than in RLP.

The long period of continued response to selection, despite relatively high amounts of accumulated inbreeding, contrasts sharply with the results from animal experiments. This difference may result from the traits selected being percentages rather than absolute values. In addition, they are storage products which apparently have little survival value to the organism.

SUMMARY

After 70 generations of selection, means of the selected character in the Illinois high protein (IHP), low protein (ILP), high oil (IHO), and low oil (ILO) strains were 215, 23, 341 and 14%, respectively, of the means of the original population (10.9% protein, 4.7% oil). Reverse selection, initiated after 48 generations of forward selection, was effective in all 4 strains. The means of reverse high protein and reverse low protein were equal after 20 generations.

Effects of changes in breeding procedure and cultural practices were evaluated by dividing the experiment into four segments: 1) generations 0 to 9, with selection based only on chemical composition; 2) generations 10 to 25, where selection for yield was imposed; 3) generations 26 to 53 in IHP and ILP and 26 to 59 in IHO and ILO with intrastrain crossing and no selection for yield; and 4) the remaining generations with the breeding procedure unchanged but nitrogen fertilizer added to the plots. Selection for yield drastically reduced progress in both IHP and ILP. The addition of nitrogen fertilizer accelerated progress in IHP.

Realized heritability values were significantly different from zero in all segments of all forward and reverse strains except the last segment of RHO. Additional evidence that selection has not exhausted genetic variation comes from comparisons of the last six generations of IHP, ILP, IHO, ILO, and RHP grown in 1970 and 1971 where significant differences among generations were found, and from significant estimates of genetic variation among half-sib families of the 65th generation.

Correlated response between oil and protein percent was found only in ILP in which a reduction in protein to 4.5% was accompanied by a significant reduction in oil percent. Protein percent increased in both IHO and ILO as a result of increased soil fertility.

Selection for chemical composition caused a marked change in kernel weight and phenotype among strains. Kernels of IHP and IHO are small and vitreous with those of IHP being the smaller. In contrast, kernels of ILO and ILP are larger with with a high percentage of soft starch. Kernels of ILO are the largest of any of the strains.

Yields of IHP, ILP, IHO, and ILO averaged 2,062; 3,454; 2,627; and 1,921 kg/ha (33, 55, 42, and 29 bu/acre), respectively, in 1970-71. Yields of the reverse strains were higher than the regular except for reverse high oil. Although comparisons of these yields with those from earlier generations are not critical because of changes in cultural practices, IHP, since 1903, was consistently the lowest yielding strain while ILP was usually the highest.

Selection for percent oil consistently altered percent germ in the kernel and percent oil in the germ. In contrast, selection for percent protein altered percent protein in the endosperm and, to a lesser degree, percent germ in the kernel.

The results of this experiment provide a vivid demonstration of the effectiveness of mild selection and recombination. In 70 generations, only about 6,000 ears have been analyzed per strain. The means of IHP, ILP, IHO, and ILO are now 12, 8, 27, and 10 S. D., respectively, beyond the mean of the original population. If 6,000 ears had been analyzed in the original population, the most extreme ear expected would have been 3.8 S. D. from the mean.

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