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Connecticut Agricultural Experiment Station
New Haven, Connecticut

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OFFICERS AND STAFF

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CONTENTS.

SUMMARY.

The results of previous investigations on inbreeding corn are reviewed to show the development of the method of selection in self-fertilized lines.

Four varieties of corn have been self-fertilized and selected for five generations. Eighty-six lines were started and twenty of these were lost or discarded.

The method of procedure was to grow three progenies in each line and self-pollinate five of the most desirable appearing plants in the best progeny each year.

A large number of clear-cut recessive abnormalities appeared during the course of the inbreeding. In all except one case these were eliminated by the fifth generation.

No significant difference in yield was found between segregating and non-segregating progenies in lines showing recessive abnormalities in the previous generation. Also lines having recessive abnormalities at the start showed no greater reduction in yield during the five generations than lines that were free from them throughout the experiment.

All lines showed a marked reduction in yield and a slowing down of the rate of growth. Although great differences were shown, no lines were as productive as the original variety. No appreciable correlation was found between the characters of the seed ear, weight of seed, size of seedling, or the appearance of the plants at pollinating time and the production of grain in the same generation.

Some correlation in certain characters was found between the first and last generations, particularly in height of plant and in per cent. of moldy ears. Less association was shown in amount of tillering and in smut infection, while in productiveness practically no relation was found, showing that good and poor yielding strains may come from productive or unproductive plants at the start.

THE IMPROVEMENT OF NATURALLY CROSS-POLLINATED PLANTS BY SELECTION IN SELF-FERTILIZED LINES.

I. The Production of Inbred Strains of Corn.

D. F. JONES and P. C. MANGELSDORF

The improvement of naturally self-fertilized plants, particularly the small grains, has gone steadily forward following the development of effective methods of procedure. In contrast to the older methods of mass selection based upon appearances, stands the system of individual plant selections chosen on the basis of the performance of their progeny, as worked out by Louis de Vilmorin in 1856 and later applied by Hjalmer Nilsson in 1891 at Svalöf in Sweden and by W. H. Hays at the Minnesota Agricultural Experiment Station in 1892. Although the early methods of applying the progeny performance test involved much unnecessary effort, the principle was sound and its extensive application has resulted in a large number of valuable new varieties of important crop plants, notably wheat and cotton. The theoretical soundness of this procedure, first applied in an empirical way, was later fully established by the re-discovery and demonstration of Mendel's Law, which postulates that a large part of inherited variability is due to the recombination of stable units. This led directly to Johannsen's genotype conception of organisms which appear alike but breed differently and those which are themselves diverse but give similar offspring.

The improvement of naturally cross-fertilized plants, reproduced by seeds, is in no such satisfactory situation. The variation brought about by Mendelian recombination makes it very difficult to have any adequate control over the heredity when inter-pollination is continually going on. Moreover, intensive selection for particular characters often results in decreasing the number of hybrid combinations and this, like all other forms of inbreeding, brings about a reduction in vigor. Any advantage which might come about from the concentration of desirable germplasm is offset by the loss of growth due to consanguinity.

Corn, a monoecious plant and wind pollinated, is almost completely cross-fertilized in every generation. This mode of pollination has brought about a condition in which a continuation of the same degree of germinal heterogeniety is necessary to maintain full vigor. The experimental results of inbreeding and crossing and their theoretical interpretation show clearly why the methods aimed at the improvement of corn in the past have been largely fruitless. Formerly the selection practiced with this plant was largely based upon the appearance of the mature ear. Investigation has shown that corn has now been brought to such a high plane of development that the correlation between the appearance of the seed and the productiveness of the crop grown from that seed is very low; so low in fact that it is often possible to get as good results from planting the poorest looking ears to be found in a field as from the choicest specimens. This is due to the fact that hybrid combinations of hereditary factors which make possible high production can not be transmitted intact and therefore the offspring of any exceptional individual can not all be equally productive.

An early appreciation of this situation following the application of experimental methods to the study of corn breeding led to the ear-to-row system in which selection was based on the performance

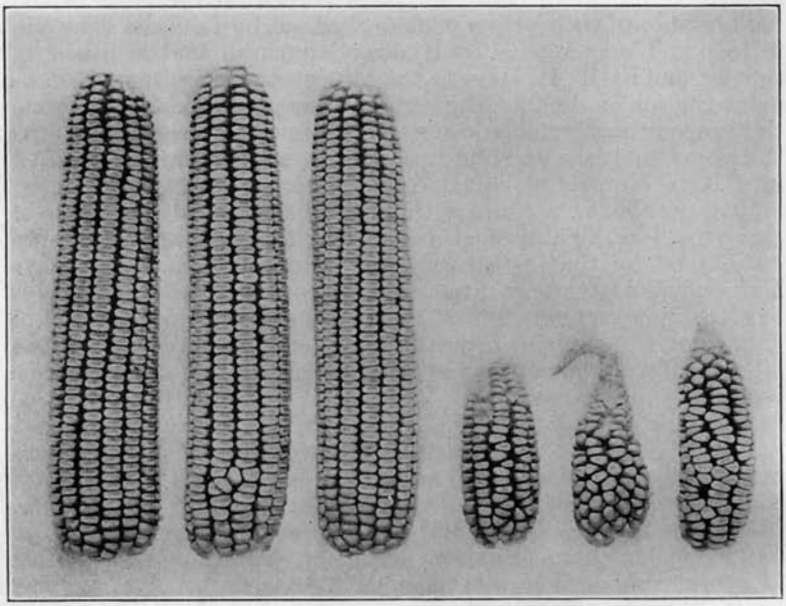

Figure 16. The seed from these large and small ears yielded the same. Their difference in size is due, not to heredity, but to the place where the plants that produced them happened to grow, one lot in a good, the other in a poor situation. This shows the complete lack of correlation in this case between the appearance of the seed ears and their performance.

of the progeny instead of the appearance of the seed parents. Although the progenies differed markedly in yield those above the average failed to maintain their high production in later generations.

In 1908 G. H. Shull outlined a method of corn breeding radically different from any previously followed. In this he called attention to the large number of germinally different types which exist in every field of corn and suggested that these could be separated out by inbreeding. Although vigor was lost by this process this was to be regained by crossing inbred strains and utilizing only the first following generation in which hybrid vigor is at its maximum. East also advocated the same method and reached the same conclusions as to the importance of hybrid vigor, as the result of independent observations on the effects of inbreeding and hybridization. The crossing of different varieties of corn had been advocated long before this by Beal at the Michigan Agricultural Experiment Station, and Morrow, Gardner and McCluer at Illinois. Two important contributions to methods for corn improvement were made by Shull and East. One was making clear the complex germinal constitution of a variety in a cross-fertilized plant such as corn and the way in which the composition of any particular individual is masked by hybrid vigor. The other was in showing that the maximum degree of hybrid vigor could be secured by first reducing the plants to homozygosity and then crossing, thereby bringing about the greatest number of hybrid combinations of hereditary units. Both East and Shull considered hybrid vigor as a physiological stimulus resulting from the condition of hybridity itself, differing from the specific action of individual hereditary factors. For this reason they stressed the importance of securing the maximum effect of hybrid vigor. The more important service of inbreeding in automatically eliminating abnormalities and serious weaknesses and in making possible the detection and isolation of the potentially most valuable germplasm was not fully appreciated at first by those who attempted to apply this method to corn improvement. For that reason the full utilization of the pure line principle was delayed until hybrid vigor was shown to be merely the expression of dominant hereditary factors. This brought out clearly and forcefully the great value of inbreeding as a means of obtaining the finest hereditary material existing in a cross-fertilized plant like corn by controlling the inheritance through the pollen parent as well as through the seed parent, and fixing this in such a way that it would not be lost. Following up this line of attack a method of corn improvement was outlined in 1920 under the general title of "Selection in Self-fertilized Lines.".* It is here proposed to review the results of inbreeding and crossing which have led to the development of this method and show how inbreeding can best be applied to the improvement of corn and other naturally cross-fertilized plants. As the application of this method is still in progress the plan is to publish the results in a series under the general heading of "The Improvement of Naturally Cross-Pollinated Plants by Selection in Self-fertilized Lines." The first of this series, submitted in the following pages, deals only with the detection and isolation of desirable hereditary qualities in corn, that is, the production of inbred strains which possess either in visible expression or in

*Jour. Agronomy, 12:77-100.

potential power those valued characters that make for increased production. Later publications are planned to deal with the testing and utilization of inbred strains of corn and the application of the same principle and method to other cross-fertilized plants.

Figure 17. Two inbred strains from the same variety that have been grown side by side for eighteen years. The difference in ability to stand erect is inherited.

The Effect of Inbreeding Upon Corn.

All of the main types of corn such as dent, flint, sweet, pop and flour corn have been inbred by self-fertilization for several successive generations. The results have been the same in general for all types. Particular attention has been given to several strains resulting from a variety of Leaming grown originally in central Illinois. Inbreeding was started by Dr. E. M. East in 1905. Four lines descending from three individual plants at the start have been continued to the present time under the direction of Dr. H. K. Hayes and later by the writers, and in 1923 they had been inbred by seventeen successive self-fertilizations. The results obtained have been reported from time to time. Particular reference is made to "Inbreeding in Corn" and the "Distinction between Development and Heredity in Inbreeding" by East, published in the report of the Connecticut Agricultural Station and in the American Naturalist, and "Heterozygosis in Evolution and in Plant Breeding" by East and Hayes in a Bureau of Plant Industry Bulletin. Later results are given in a bulletin of the Connecticut Agricultural Station under the title of "The Effects of Inbreeding and Crossbreeding on Development" and the "Attainment of Homozygosity in Inbred Strains of Maize" in Genetics by the senior writer. As the method of selection in self-fertilized lines

Table I.

Yield and Height of Four Inbred Leaming Strains of Corn Self-Fertilized Seventeen Generations.

No. of Gen. Selfed	Strain A		Strain B		Strain C		Strain D	
	Yield Bu. per Acre	Height Inches	Yield Bu. per Acre	Height Inches	Yield Bu. per Acre	Height Inches	Yield Bu. per Acre	Height Inches
0	74.7	117.3	74.7	117.3	74.7	117.3	74.7	117.3
1	42.3	....	60.9	....	60.9	....	59.1	....
2	51.7	....	59.3	....	59.3	....	95.2	....
3	35.4	....	46.0	....	59.7	....	57.9	....
4	47.7	....	63.2	....	68.1	....	80.0	....
5	26.0	76.5	25.4	81.1	41.3	90.5	27.7	86.7
6	38.9	....	....	....	....	....	....	....
7	45.4	85.0	39.4	....	....	....	41.8	....
8	21.6	....	47.2	83.5	58.5	88.0	78.8	96.0
9	30.6	78.7	24.8	....	....	....	25.5	....
10	31.8	82.4	32.7	84.9	19.2	86.9	32.8	97.7
11	35.1	79.7	42.3	78.6	37.6	83.8	46.2	103.7
12	24.5	77.0	27.2	80.3	20.4	85.2	49.6	100.4
13	26.9	85.5	29.0	83.7	25.1	80.6	25.8	85.3
14	23.6	87.3	38.3	86.9	36.3	87.8	35.2	94.0
15	21.1	85.4	33.4	89.9	30.0	98.2	33.6	99.6
16	17.6	76.1	24.6	89.1	25.3	94.6	29.8	97.7
17	....	....	27.8	91.7	16.9	88.9	19.8	88.4

has been the direct outgrowth of these investigations as to the effects of inbreeding, a brief resumé of the results obtained to date will be given here.

The method of inbreeding followed in the earlier experiments was to self-pollinate a number of plants at random and use one of these as the progenitor for the following generation. Such a family descending from a single self-fertilized plant in each generation is called a line or strain. The yield of grain and height of plant of four lines from Leaming during seventeen successive self-fertilized generations compared to the non-inbred variety are given in Table I. The four lines *A*, *B*, *C*, *D*, were derived at the start from three different plants. One of these was separated in the third generation into two lines, *B* and *C*. These have been continued separately since. Other lines were started from the same variety but have since been lost on account of failure to secure self-pollinated seed. In some cases this loss has been accidental, but for the most part these strains were maintained previous to their extinction with great difficulty and showed a much greater reduction in growth and vigor than the other strains which survived.

Although there is wide variation in yield of grain and height of plant from year to year the general direction is downward. After the ninth generation size and productiveness have remained on about the same level. The original variety yielded at the rate of eighty-eight bushels per acre the year it was first self-fertilized. In 1916 seed of the same variety was obtained from the original source and grown in comparison with these strains, then in the ninth or tenth generation. On account of its change to a new location under conditions to which it was not as well adapted as the inbred strains, which had been grown there for many years, no strict comparison can be made. In spite of their possible advantage the inbred strains were only from one-half to one-third as productive and were also noticeably reduced in height.

This decrease in yield which results from a reduction in size of all parts of the plant and a lessening of the growth rate has so far been the universal result of inbreeding corn as far as known to the writers. Several hundred self-fertilized strains have been grown long enough to bring this out clearly. Accompanying the lessening of productiveness and growth vigor there has been a reduction in variability. From a variety that showed the usual variation in height, color of silks, glumes and leaf sheaths, number of ears, position of the ear and other details in all parts of the plants there resulted in the four self-fertilized lines a marked uniformity among all of the plants within each line. This similarity in type became noticeable in the earlier generations of inbreeding, and after seven or eight successive self-fertilizations every plant in any one line was as much like every other plant in that line as any two plants in a naturally self-fertilized species, such as wheat or tobacco, from seed from the same individual. In other words, the variability that resulted from the recombination of hereditary factors was in time eliminated.

Figure 18. Two inbred strains from the same variety of flint corn, one with many tillers and the other without any.

Where the original variety had some plants with colored silks and others with uncolored, some of the lines now have all their plants with red silks while in others all the silks are green. In some lines the foliage on all the plants is a bright glossy green, in others a dull bluish green. All the plants of one of the lines remain green and stand firmly erect throughout the season while in other lines the foliage turns yellow towards the end of the growing season and in still another the plants frequently go down on account of a weak root system. Differences in susceptibility to smut are shown in these four strains as brought out in table II. In every detail of structure of the plant, including tassel and ears, all the individuals of one line are remarkably alike and noticeably different from the other lines. Some of these differences are shown in the accompanying illustrations, figures 17, 18 and 19. The uniformity within the line and the differences between the several lines are brought out statistically in tables III to VI, which show the height of plant, length of ear, number of nodes and rows of grain on the ear for the original variety and the four strains derived from this variety.

TABLE II.

Per cent. of Plants Showing Smut Infestation in Four Inbred Leaming Strains.

Strain	1917	1918	1919	1920	1921	1922	1923	Ave
A	.3	.7	1.9	14.3	15.2	3.0	.0	5.1
B	9.8	25.9	8.6	32.8	50.0	27.3	69.0	31.9
C	.5	9.1	4.1	6.0	13.8	17.5	52.7	14.8
D	.0	1.0	1.4	25.0	4.1	2.2	.7	4.9

During the early generations of self-fertilization various forms of abnormalities appeared. The most frequent of these are seedlings wholly or partially lacking in chlorophyll, various types of striped plants, golden plants, dwarfs, plants with ears showing many poorly developed and aborted seeds, and others with sterile tassels and ears. These are a few of the more strikingly aberrant types. Some of these are able to produce seed and when self-fertilized come true to their abnormal condition. Others are wholly incapable of reproduction and are eliminated, but the inbred strains in which they appear may continue to produce them regularly as part of their offspring in the following generations. After several generations these abnormalities are usually no longer produced and the remaining plants are all normal in type although reduced in size and in rapidity of growth. Many of the abnormal forms which appear in large numbers in the inbred families are occasionally seen in fields of corn which have never been artificially self-fertilized. Obviously, inbreeding is not responsible for their creation. They are recessive in mode of inheritance; that is, when crossed with other plants the following generation is all normal but the abnormality reappears in the subsequent generations.

Figure 19. Differences in height of two inbred strains from the same variety self-fertilized four generations and selected for vigor and productiveness but not for height.

TABLE III.

Frequency Distribution of Height of Plant of Leaming Corn and Four Inbred Strains Derived from It.

	Height of Plant in Inches.																				
	58.5	63.5	68.5	73.5	78.5	83.5	88.5	93.5	98.5	103.5	108.5	113.5	118.5	123.5	128.5	133.5	138.5	143.5	N	A	C. V.
Variety	..	..	..	..	..	..	..	5	7	17	33	37	32	33	31	13	4	1	213	117.3±.48	8.81±.29
Strain A	2	2	2	24	48	37	4	..	..	..	..	..	..	..	..	..	..	..	119	78.6±.33	6.78±.30
Strain B	3	6	10	11	24	42	59	39	7	..	..	..	..	..	..	..	..	..	201	84.6±.41	10.22±.35
Strain C	..	1	2	9	11	26	21	21	12	2	..	..	..	..	..	..	..	..	105	86.7±.54	9.46±.44
Strain D	..	..	..	..	2	2	18	56	79	50	8	4	..	..	..	..	..	..	219	97.9±.26	5.91±.19

TABLE IV.

Frequency Distribution of Length of Ear of Leaming Corn and Four Inbred Strains Derived from It.

	Length of Ear in Inches.												
	2	3	4	5	6	7	8	9	10	11	12	N	A	C. V.
Variety	..	..	5	15	32	65	61	37	10	2	1	228	7.5±.06	19.07± .63
Strain A	..	4	17	69	24	..	..	..	..	..	..	114	5.0±.04	14.18± .65
Strain B	8	31	88	82	2	..	..	..	..	..	..	211	4.2±.04	19.94± .68
Strain C	1	4	16	26	32	6	..	..	..	..	..	85	5.2±.08	20.17±1.08
Strain D	..	3	17	31	58	81	27	2	..	..	..	219	6.3±.05	19.00± .63

TABLE V.

Frequency Distribution of the Number of Nodes of Leaming Corn and Four Inbred Strains Derived from It.

	Number of Nodes.													
	8	9	10	11	12	13	14	15	16	17	18	N	A	C. V.
Variety	..	..	2	5	23	49	79	60	11	12	4	245	14.1 ± .06	10.07 ± .31
Strain A	..	..	..	5	21	66	26	1	..	..	..	119	13.0 ± .05	5.97 ± .26
Strain B	1	2	11	33	103	51	8	1	..	..	..	210	12.0 ± .05	8.21 ± .27
Strain C	..	1	2	15	30	44	16	..	..	..	..	108	12.5 ± .07	8.18 ± .38
Strain D	..	1	1	48	113	59	9	..	..	..	..	231	12.1 ± .04	6.77 ± .21

TABLE VI.

Frequency Distribution of the Number of Rows of Grain on the Ear of Leaming Corn and Four Inbred Strains Derived from It.

	Number of Rows of Grain on the Ear.										
	12	14	16	18	20	22	24	26	N	A	C. V.
Variety	3	14	51	75	52	21	7	4	227	18.4 ± .12	14.22 ± .46
Strain A	39	70	3	2	..	..	..	..	114	13.4 ± .08	8.94 ± .40
Strain B	..	..	1	22	56	78	43	6	206	21.5 ± .09	9.38 ± .31
Strain C	..	24	42	18	1	..	..	..	85	15.9 ± .11	9.26 ± .48
Strain D	11	90	86	26	2	..	..	..	215	15.2 ± .07	10.50 ± .34

In ordinary fields of corn they are generally kept out of sight by continual crossing with normal types which are dominant. Plants carrying such factors for abnormality, when self-fertilized, produce them in approximately one-fourth of their progeny. Some of the normal plants in the same progeny carry the abnormality and some do not. Sooner or later, progenitors are used which do not carry any of these striking abnormalities, after which they cease to appear.

The rate at which reduction in growth takes place and the final size and productiveness of the several lines, after the reduction comes to an end, vary in different lines. Of the four Leaming strains the *D* line has regularly been taller and larger and has yielded more than the others. The rate of reduction has been nearly alike in all of the four lines although *A* was reduced in yield somewhat more quickly than any of the others. The attainment

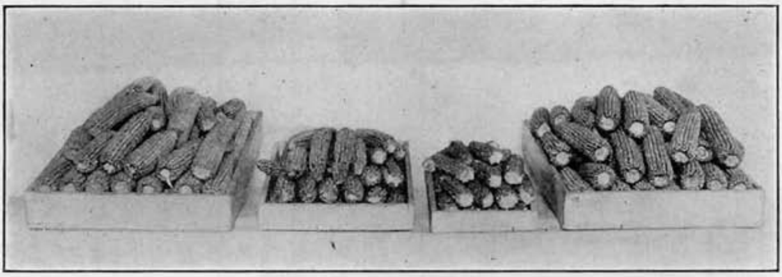

Figure 20. Comparative production of a variety of Leaming corn, two inbred strains derived from this variety, and their first generation hybrid. Grown in adjoining rows, they yielded 96, 32, 20 and 115 bushels per acre respectively.

of uniformity may also proceed at a different rate, depending upon the degree of heterozygosity of the plant chosen as progenitor. Some strains remain variable for many generations while others become uniform in nearly every feature after a few generations of self-fertilization.

From the foregoing facts it is obvious that inbreeding is a process of sorting out. From a mixture of many genetically different individuals all varying in hereditary composition and in heterozygosity any number of homozygous lines can be ultimately obtained, each differing to a greater or less degree from every other. A naturally cross-fertilized species is thus changed into an artifically self-fertilized species. In uniformity and constancy these artificially inbred plants are quite comparable to naturally self-fertilized species, with the important difference that in corn they are markedly reduced in size and vigor.

Result of Crossing.

The vigor which is lost by inbreeding is at once restored when two self-fertilized lines descending from different plants at the start

Figure 21. Two inbred strains and their first generation hybrid showing differences in time of flowering.

are crossed. This is shown in figure 20. Here the ears produced by the original non-inbred variety are shown in comparison with the ears produced by two lines self-fertilized 12 generations and the first generation hybrid between these two lines. An equal number of plants of the four lots were grown in adjoining rows and yielded 96, 32, 20 and 115 bushels per acre respectively. A comparison of a large number of first generation crosses between inbred strains derived from the same variety showed that the yield of the hybrids was increased 180 per cent., height of plant 27, length of ear 29, number of nodes 6, and rows of grain on the ear 5 per cent. above the average of their inbred parents.* From this it is seen that size characters such as height of plant and length of ear are affected more noticeably by hybrid vigor than the number of parts, such as nodes and rows of grain on the ear, while yield, which sums up

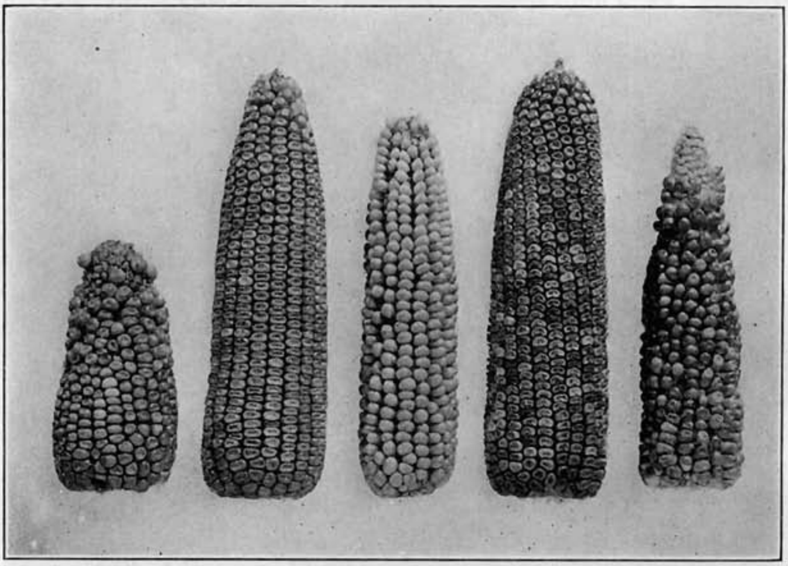

Figure 22. Representative ears of three inbred strains of dent corn and two first generation hybrids resulting from the crossing of the two adjoining types, harvested at the same time to show the difference in maturity.

the entire growing capacity of the plant, is increased more than anything else. In other words hybrid vigor has much the same effect as favorable environmental factors. Fertile soil, good season and careful cultivation influence the growth of the corn plant. Under these conditions corn grows taller, the ears are larger and the production of grain is much greater than under the less favorable conditions, while the number of nodes or the rows of grain on the ear are not so much changed.

*"The effects of inbreeding and crossbreeding upon development." Connecticut Agric. Exper. Station Bull. 207.

Another noticeable effect of crossing inbred strains of corn is that of hastening the time of flowering and maturing. Figure 21 shows two inbred strains in which the tassels are just beginning to appear. No silks are out. The first generation hybrid of these two strains in the center is shedding pollen from nearly all of the tassels and the silks are well out on many of the plants. Representative ears of three inbred strains and first generation hybrid ears resulting from the cross of the two adjacent strains are pictured in figure 22. All were picked at the same time and show the greater maturity of the hybrid ears.

All of the combinations of inbred strains have shown increased

Figure 23. A first generation hybrid showing the uniformity in height and in tassel type. The two inbred parental strains are in the adjoining rows at the left.

growth and yield whether the parental strains come from the same original variety or from different varieties. Some combinations have yielded more than others. A few have been better than others in many respects. Crosses between strains from different varieties have not been conspicuously better than crosses within the variety although no extensive test of this point has been made. Furthermore, no reliable comparison of the yield of the hybrids with the original variety can be made because this variety is not well adapted to the local conditions in which the self-fertilized lines have been grown for many years. Kiesselbach reports the average yield of seven first generation hybrids tested two years as 52 bushels per acre in comparison with 42 bushels for the original variety. This is an increase of 24 per cent. The highest yielding hybrid produced 59 bushels or an increase of 40 per cent.

The most noticeable and important feature of the first generation hybrids between fixed inbred strains is the even growth, similarity in size and structural details and uniform production of all plants where the growing conditions are equal. This is shown for height of plant and tassel type in figure 23. Barring accident every plant is like every other plant. They grow to the same height. All ears are borne usually at the same node. The tassels and silks appear at the same time and the plants all ripen within a few days of each other. The fact that every plant produces a good ear is a most important factor in making crosses between strains so productive. In ability to yield from every plant and in uniformity of ripening, these first generation corn hybrids are equal to any naturally self-fertilized crop such as wheat and tobacco or any vegetatively propagated plant as potatoes and sugar cane. Since corn is very susceptible to damage by unfavorable weather at pollinating time, the uniformity in flowering may be undesirable particularly in those regions where hot dry weather is a frequent occurrence at this critical time. For that reason some other method of utilizing inbred strains may prove to be more practicable. This will be considered more fully in later publications. It is sufficient here to point out that in these first generation hybrids we have a new kind of corn which in many important respects is radically different from the mixtures of hybrids of varying degrees of heterozygosity now constituting an ordinary field of corn.

An Interpretation of Hybrid Vigor.

The observations of gardeners and animal husbandmen have led to a general conviction that crossing somewhat different but related plants or animals usually results in a greater growth. Many instances of this phenomenon of hybrid vigor, in which the offspring excel both parents have been noted in the higher plants and in mammals, birds, insects and some of the lower forms of animals. Larger size or more rapid growth usually results when the parents are visibly different in some respects but are sufficiently related to produce fertile offspring. Many notable cases of hybrid vigor also occur in wider crosses where the offspring are partially or wholly sterile. This is well illustrated by the mule, which is sterile. A similar wide cross in plants is the combination of the radish and cabbage in which the hybrid makes a luxuriant growth but sets no seed. Some species crosses show no increased vigor but on the other hand may be extremely weak. East and Hayes have given several illustrations of tobacco hybrids which are barely able to live and make only a weak growth. Many crosses of different species in animals and plants do not develop normally. Hybrid weakness as well as hybrid vigor must be taken into consideration although this is not be to expected in crosses that are fertile.

After the limits of physiological compatibility are reached cross-fertilization cannot be accomplished. A series can therefore be arranged as follows: (1) Crosses between organisms which are so nearly alike in germinal constitution that no increased growth

Figure 24. Crossed corn showing vigorous growth.

results. (2) Crosses between germinally diverse but closely related organisms that grow to a larger size and at a more rapid rate and are fully fertile. (3) Sterile crosses between more distantly related organisms which are extremely vigorous. (4) Sterile crosses which are weak and often abnormal. (5) Crosses which cannot be made on account of the germinal difference in the forms united.

Hybrid vigor in domestic animals and cultivated plants most frequently results when breeds or varieties of different type are brought together. Thus it is a common practice to cross the bacon and lard types of hogs or the mutton and wool breeds of sheep to secure some of the advantages of both parental races. Dent and flint varieties of corn when crossed usually give greater increases in yield than crosses within either type. In these diverse crosses many of the desirable features of both parental races are brought together. How this works is well illustrated in the cross of a "golden" type of corn which is deficient in chlorophyll with a "dwarf" as shown in figure 26. The plants resulting from this cross are tall, normally green and quite vigorous and productive. In this particular case one parent contributes normal stature and the other normal chlorophyll. Both these characters are dominant over the recessive condition so that all the hybrid plants

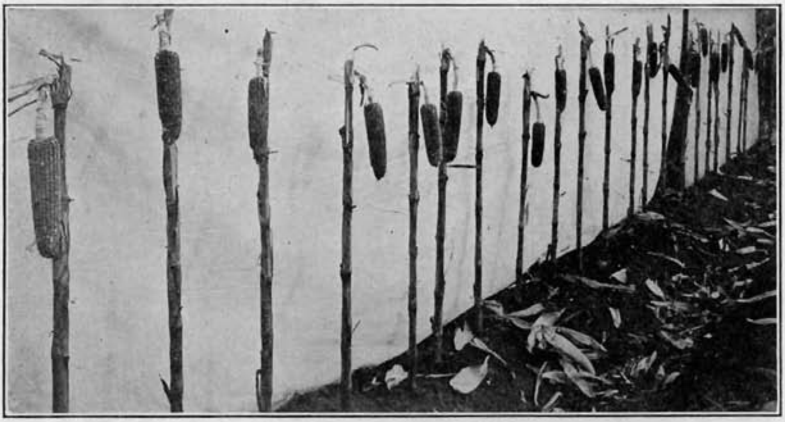

Figure 25. It is the uniform production of a good ear on every plan- that makes the first generation hybrids between inbred strains so product tive.

are alike in their tall stature and green color. Another case is shown in figure 27 of two dwarfs which are genetically different and which, when crossed, give a tall, vigorous hybrid. One of the dwarfs lacks something essential to normal height and all the plants are alike as long as they are not out-crossed. The other dwarf is lacking in some other essential factor present in normal corn. When these two small plants are combined each type supplies what the other lacks so that the result is normal stature in all the hybrid plants the first year after crossing. These illustrations of the result of crossing are extreme cases which show how conspicuous abnormalities are suppressed by crossing so that the hybrid offspring are able to make a greater growth than either parent. The same situation in principle exists in all crosses from which hybrid vigor ensues. Different organisms possess different hereditary qualities. When brought together there is always a tendency for the hereditary factors which make for greater growth vigor to dominate the factors for lesser growth. The bringing

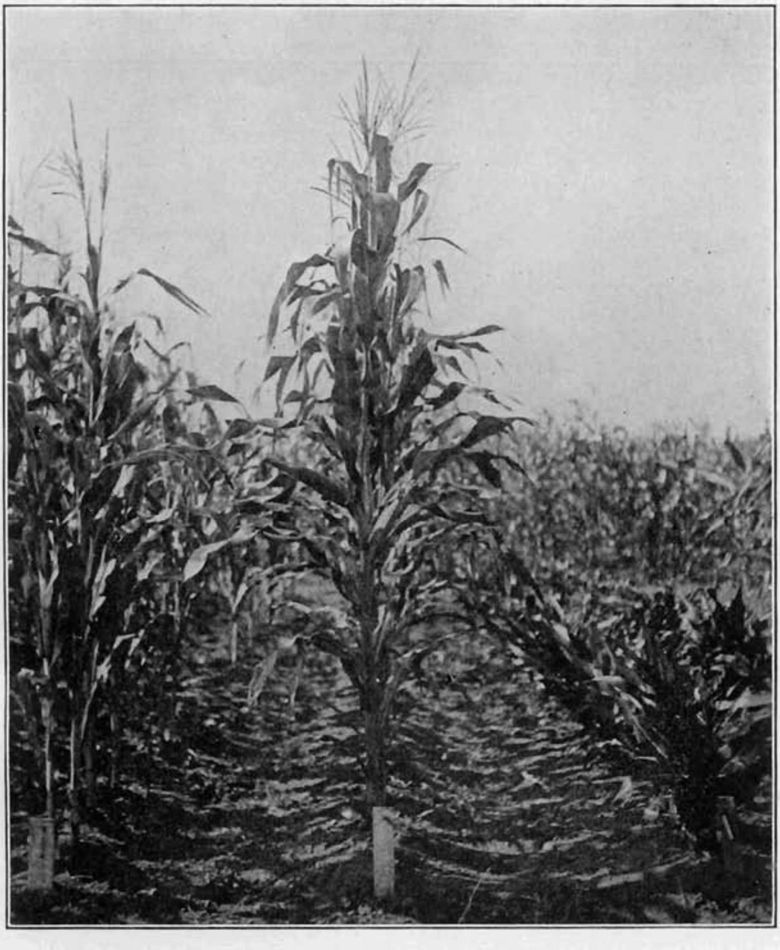

Figure 26. The result of crossing a golden, liguleless type, on the left, with a green dwarf on the right. The hybrid, in the center, has tall stature, normal foliage and green chlorophyll due to dominant factors contributed by each parent.

together of the best of both parents in this way gives the hybrid offspring a temporary advantage over either parent in the first generation following the cross. Recessive weaknesses are continually occurring as mutations as shown by the many controlled observations on the fruit fly and other forms of life. In cross-fertilized organisms, and particularly in domesticated animals and plants, crossing keeps these covered over and out of sight by combining them with normal factors. Many of these recessive weaknesses are not distinct and visible characters as are the

Figure 27. Two genetically different dwarf types give tall plants when crossed, due to the fact that the normal growth factor which each lacks is supplied by the other.

chlorophyll deficiency or dwarfness in corn but nevertheless they weaken the organism in some way. When such crossbred races are inbred, the heterozygous combinations are reduced and the resulting individuals which are homozygous to a greater and greater degree, as the inbreeding is continued, show the recessive weaknesses and are either unable to reproduce themselves or are reduced in size and rate of growth to a point below that of the original stock. The inbred individuals each receive some of the hereditary factors for vigorous growth. Some receive more than others as a chance allotment and are therefore better able to survive the inbreeding process. Others are so weakened that they perish. On account of the way in which the hereditary mechanism operates it is extremely improbable that any one individual will receive all the more favorable growth factors, and in actual practice inbred strains of corn are all reduced by inbreeding. It is theoretically possible to obtain individuals which possess an unusually large share of the more favorable growth factors or even all of them and for that reason show no reduction from inbreeding. Darwin obtained self-fertilized races of *Ipomea* and *Mimulus* which were more vigorous than the naturally cross-fertilized variety at the start. Cummings reports self-fertilized strains of squash that are as productive as the original variety and much more uniform in type. King has obtained inbred rats after long-continued brother and sister mating that are fully as vigorous as the material with which she started. The fact that no such result has been obtained with corn shows how dependent this plant has become upon cross-fertilization to maintain production.

The Transitory Nature of Hybrid Vigor.

The increased growth resulting from crossing is quickly lost in the following generations when the hybrid individuals are bred among themselves or again inbred. In other words, hybrid vigor is a temporary manifestation which ordinarily cannot be fixed and made permanent in sexually reproduced offspring. The reason for this is readily appreciated when the illustrations previously given are followed into the later generations. The cross of the golden and dwarf corn gives all normal tall green plants in the first hybrid generation. Seed from these hybrid plants, either selfed or inter-crossed, always gives in the next generation all the possible combinations of characters that went into the cross. In this particular case the golden plants also lacked the ligule which is the small extension of the leaf sheath surrounding the stalk above the leaf blade. Liguleless plants hold their leaves in a characteristically upright position close to the stalk. In the second generation of this cross of liguleless golden by dwarf, eight different kinds of plants are produced. These are shown in figure 28. Due to the recombination of Mendelian units, this generation is extremely variable, and while some of the tall, green, liguled plants may be as vigorous and productive as the first crossed plants this generation as a whole averages much less productive. By further inbreeding, eight distinct pure-breeding combinations of these three characters can be obtained and within each type still further minor differences could be established. Crossing any two of these types gives increased growth and restores the normal condition provided the factors for normal growth are all present in one or the other type.

In the same way the vigorous and productive crosses between inbred strains of corn fall off in size and yield in the second generation and are much more variable. This always results whether the first crossed plants are self-fertilized or are inter-crossed among themselves. If the inbred strains are uniform and fixed in their type the first generation hybrid plants are germinally all alike so

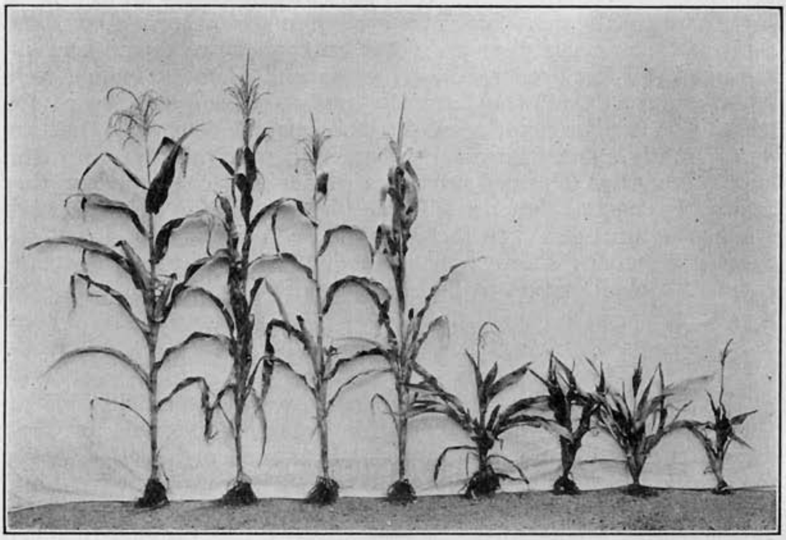

Figure 28. The second generation offspring from the crossing of golden liguleless by dwarf. Eight different combinations of these three characters are obtained by Mendelian segregation and recombination.

that it is easily understood why self-fertilization and inter-crossing give the same result. To test this out two inbred strains were crossed after 14 generations of self-fertilization. A number of the hybrid plants were self-fertilized and an equal number were inter-pollinated. The seed of these two lots was planted in alternate rows, replicated three times. The self-fertilized plants averaged $76.2 \pm .57$ inches in height in comparison with the intercrossed plants which averaged $73.8 \pm .70$. In production of grain they stood respectively 22.2 ± 1.2 and 22.0 ± 2.4 bushels per acre. In neither case are the differences significant.

Inbreeding after Crossing.

When the second generation plants are allowed to intercross naturally no further reduction in vigor is expected. Variability and yield should remain at the same level thereafter until natural or artificial selection eliminates certain strains. But when the second generation plants are self-fertilized there is a further reduction in size, and if the inbreeding is continued the decline in size and vigor and in variability proceeds in approximately the same way as when the parental strains were first inbred. This is shown in figures 29, 30 and 31.

In this demonstration of inbreeding after crossing, two inbred strains, self-fertilized for eight generations, were crossed and the first generation plants again self-fertilized. In the second generation a single plant was again chosen as the progenitor and pollinated in the same way, and this was continued for eight successive

Figure 29. The result of inbreeding after crossing. Two inbred strains at the left, their first generation hybrid adjoining, followed by seven successive generations self-fertilized.

generations. Seed was saved from each year's selfing up to the fifth generation. Since corn seed will not retain its germination satisfactorily for more than six years, single plants were again self-fertilized the fifth year in each generation and this seed was used from then on. All eight inbred generations were grown in 1923 along with the two parental strains as shown in the accompanying illustrations. This demonstration has been grown each year since the original cross was made and the yields obtained in the different years are given in table VII. Production has varied rather widely from season to season and from generation to generation. This is due in part to the character of the individual plants chosen for progenitors. A very noticeable drop takes place from the first to the second generation amounting to over 30 per cent. as an average of the six years. Kiesselbach tested the first and second generations of eight hybrid combinations of different strains during two seasons and obtained an average of 52.2 and 27.8 bushels per acre respectively for the two generations, to be compared with 41.7 bushels for the original corn from which the inbred strains were obtained. He secured his seed for the second generation by pollinating several first generation plants with composite pollen from 15 sib plants. The reduction from the first to the second generation of nearly 50 per cent. is even greater than in our case where the plants were self-fertilized. Kiesselbach also grew a third generation from seed of interpollinated plants. The comparative yields obtained for the first, second and third generations were 51.5, 29.4, and 25.6 bushels per acre. The reduction from the second to the third as would be expected from this mode of pollination is small compared with the drop from the first to the second. Continued inter-pollination should cause no further decrease in yield unless particularly unfavorable strains are isolated.

The average height of these successive self-fertilized generations compared with the first generation hybrid and the parental strains is shown graphically in figure 32. There is a continued

TABLE VII.

The production of grain in bushels per acre, of two inbred strains of corn and their hybrid and the F_1 to the F_8 generations successively self-fertilized.

Year Grown	P_A	P_B	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
					Generations					
1917	22	6	65	56	....	....	....	....	....	....
1918	27	24	121	128	15	....	....	....	....	....
1920	16	28	128	48	35	29	10	....	....	....
1921	20	13	73	55	49	33	15	23	....	....
1922	20	26	160	83	74	68	49	36	23	....
1923	13	21	61	45	41	47	16	23	26	27
Ave.	20	20	101	69	43	44	23	27	25	27

reduction in each generation, but the decrease is much less during the last three generations than in the first four. From the first to the fifth generation there is a decline of 27.2 inches in stature and from the fifth to the eighth 8.6 inches. The rate of growth as measured by the daily gain in height is also steadily reduced as shown in figure 33, the decline being greater during the first stage of inbreeding than in the last. The differences between the last two generations in all measurable characters, including yield, height, length of ear and rate of growth, are so small that it seems evident that the reduction in size and vigor is rapidly approaching an end. The last two generations are so similar in appearance that they cannot be distinguished in the field. In tassel type, foliage character, position of the ear on the stalk, and in the size and conformation of the ears these two generations are practically identical.

The reduction in variability from the first to the eighth generation was very noticeable in the field. One of the parent strains has green silks, the other red. The first generation hybrid plants all had red silks. The second, third and fourth generations segregated for this color while the remaining generations were all uniformly colored. Height of plant, position of the ear on the stalk, form of tassel and all structural details were noticeably uniform in the parents and the first hybrid generation. The plants in the generations from the second to the fifth were quite variable but later became more and more uniform until in the last two generations they showed as little variation as either of the parental strains.

The inbred strain which resulted from this second period of self-fertilization differs from both parental strains. In tassel, ear, and character of the foliage it is quite unlike either but is noticeably susceptible to smut like one of the parents. In other words,

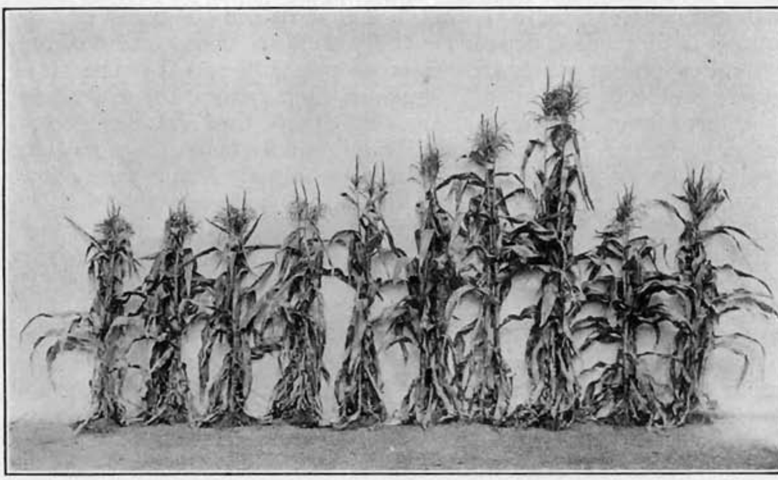

Figure 30. Inbreeding after crossing. Representative plants from the generations shown in figure 29.

Mendelian recombination has taken place so that the details of structure are altered. Apparently this inbred strain has about the same number of favorable growth factors, and for that reason it is no better or no worse than the parental stocks that went into the vigorous and productive hybrid from which the new strain was derived a few generations before.

For all practical purposes the reducing effect of self-fertilization in this particular case has ceased at the sixth inbred generation. This closely parallels the course of events when the parental strains were first inbred. Theoretically the loss of vigor follows the rule of halving the remaining difference in each generation. If we take an individual heterozygous for a single Mendelian pair of factors such as *Aa* we expect in the next generation fifty per cent. of the plants homozygous for this pair of factors and having the composition *AA* or *aa*; the other fifty per cent. will on the average still be heterozygous for this factor pair; i. e. *Aa* in composition. In choosing a single self-fertilized individual for the progenitor the chances are even that it will be homozygous or heterozygous. This holds for any number of factor pairs and since each pair when once alike must remain so thereafter in self-fertilization the number of mixed pairs is steadily reduced by half in each generation. Starting with an individual 100 per cent. heterozygous, the following generations would be on the average 50, 25, 12.5, 6.25, 3.125, 1.5625, etc.

Naturally the progeny of any heterozygous individual will vary greatly in composition. Some will be nearly or completely homozygous while others will be nearly or completely heterozygous with respect to all factor pairs. For that reason the result of any process of inbreeding depends entirely upon the composition of the individual plants which are chosen as progenitors. It is theoretically possible to obtain individuals in each generation which are as heterozygous as their parents and others that are completely homozygous. For that reason inbreeding may cause no reduction in size, vigor or variability, or complete reduction may take place in a single generation. The chances that such a result will be obtained, however, are extremely remote. Actually the reduction follows the rule of halving the remaining difference very closely so that it is evident that a very large number of factors play a part in hybrid vigor. How many such factors there are, we have no way of estimating at the present. Many factors which bring about visible differences possibly have no effect upon vigor but apparently the number of them which are essential to normal development in corn is exceedingly great.

The Attainment of Complete Homozygosity.

Whether complete fixity of type, absolute homozygosity, is possible of attainment by continuous self-fertilization has been previously discussed. (Jones 1924.) The experimental results show that small germinal differences may remain after many generations of inbreeding. Two lines separated from one in the third generation and then continued separately for several generations gave a marked increase in size when crossed, although not as great as in the case of lines separated at the beginning, showing that two self-fertilizations had not produced much uniformity in germinal constitution. The four original Leaming strains were continued as single lines up to the eighth generation. At that time they were all remarkably uniform and apparently fixed in their type. Then each line was separated into two lines which were continued separately thereafter for eight or more additional generations. At that time two of the paired lines had remained exactly alike. No visible differences in any respect could be seen.

One of the paired lines differed only in color of the seeds, one being noticeably brighter in color in some seasons. As the growing conditions were alike for all plants this slight difference can not be accounted for in any other way than as an heritable difference. The other paired line differed noticeably in many respects. One of the members was taller, the leaves were broader and lighter colored and the ears were larger, the seeds broader and duller in color.

Crossing these paired lines gave significant increases in all measurable characters in the one strain whose paired lines were visibly different. The other strains all showed slight but apparently significant increases in some characters. The two strains whose paired lines showed no visible differences were again tested after fourteen generations of self-fertilization in the following way. The two strains which were distinct from the beginning were crossed and gave the usual vigorous and uniform hybrid plants. A

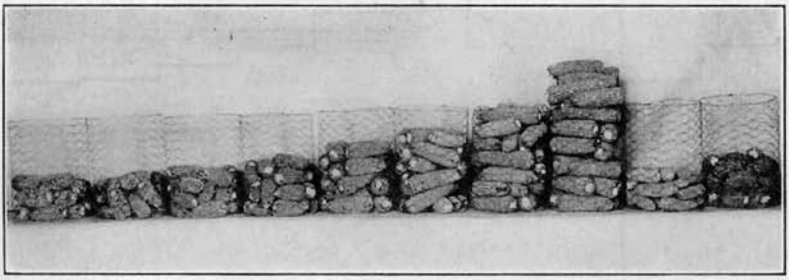

Figure 31. Inbreeding after crossing. The production of grain from the plants shown in figure 29.

number of these were self-fertilized and an equal number were inter-pollinated by sib plants. A careful test failed to show any differences in size or productiveness in the plants grown from these two lots of seed. If the parental strains were not germinally alike within themselves, intercrossing the first generation hybrid plants would not cause such a decrease in heterozygosity as self-fertilization. The fact that no difference was shown indicates that the parental strains were completely homozygous for all factors which influence growth vigor. However, this test is not a very delicate one and final proof awaits the crossing of the paired lines which have been separated in the seventeenth generation and will be carried along for several additional generations.

Mutations in Corn.

Complete homozygosity may be impossible to attain because of spontaneous variations, mutations, occurring from time to time.

During the seventeen years in which the four inbred Leaming strains have been under observation only two apparent germinal changes have been recorded. Until a fairly high degree of uniformity was reached, after six generations, various abnormalities occurred singly or in greater numbers in the rather small progenies that were grown. Presumably these were, at least in the great majority of cases, merely segregations from a heterozygous complex. But new characters appearing after uniformity is obtained which have not been noted previously have every indication of being mutations. Two such have been observed in different lines. One produced in the thirteenth generation a single self-pollinated ear segregating for defective seeds. All of the lines had been examined for the new character during three previous generations, without noting anything of this kind, and since the character

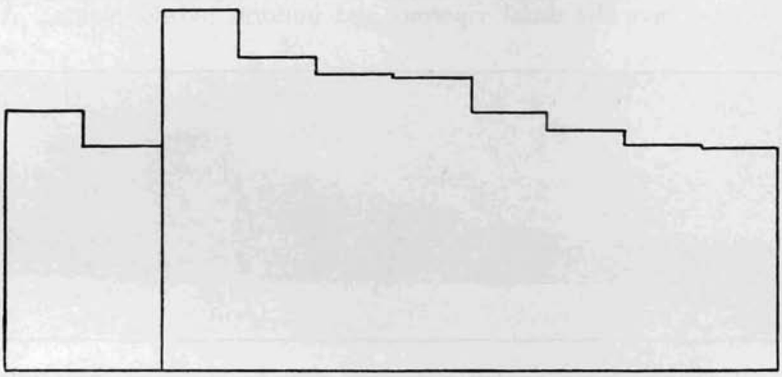

Figure 32. Graph showing the height of the two parental strains and the generations from the F_1 to F_8.

segregated as a single Mendelian recessive when out-crossed, there is every reason to assume that a germinal change took place shortly before its appearance. Among approximately a thousand plants of another line, self-fertilized more than ten generations, which has always produced white cobs, four ears were found with light red cobs. The cobs of this strain are flattened and the plants are otherwise easily identified. The red cob plants were examined at harvest and noted to be typical for the strain in all respects except cob color. Neither of these changes could have been due to out-crossing. Stray pollen from any outside source immediately results in vigorous plants twice as large as the inbred plants ever grow and the crossed plants are completely changed in type. Since the mutant plants were in other respects typical plants of the strain and were no larger they could not have resulted from out-crossing.

Two additional changes have occurred in other inbred material such that they have every indication of being recent germinal alterations. One strain after five generations produced for the first time striped, variegated plants which bore no pollen or seed. They occured in later generations in about 25 per cent. of the offspring from normal plants. Another strain after nine generations gave small narrow-leaved dwarf plants which were quite distinct from the normal plants. They produced a small amount of pollen and when out-crossed to normal plants they reappeared in later generations showing that the change was heritable.

These four apparent mutations are all that have been noted in a large number of uniform strains which have been under observation for many years. Hayes and Brewbaker record the production of chlorophyll deficient seedlings in four lines out of 953 which had

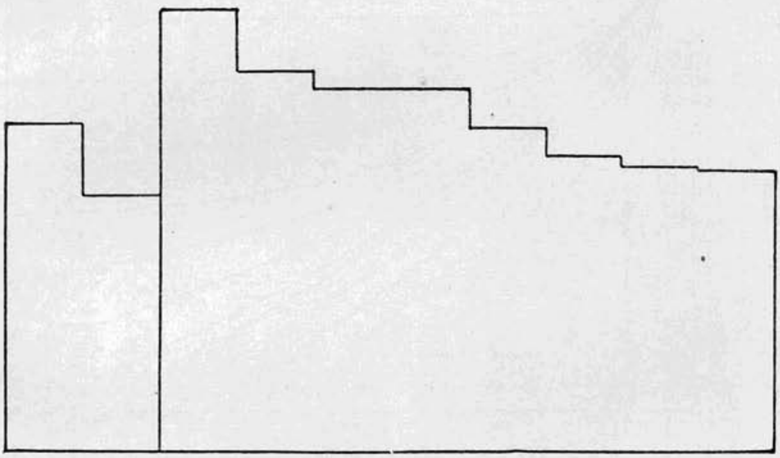

Figure 33. Graphs showing rate of growth (average daily gain in height) for the same generations as in the preceding illustrations.

not shown such abnormalities previously. In these cases the appearance of the abnormalities may have been due to delayed segregation, since the lines had not been reduced to uniformity and constancy. While it is evident that corn does mutate, the frequency of these changes is so low that inbred strains, when once reduced to uniformity, are stable for all practical purposes. Some care will be needed to maintain self-fertilized lines true to type, and when recessive abnormalities appear those progenies which show them will have to be discarded.

The Value of Inbreeding.

This review of the effects of inbreeding and crossing upon corn has been given in considerable detail because the facts learned from these investigations form the basis for the method of improvement by selection in self-fertilized lines. In the inbreeding experiments just described no selection of superior individuals to perpetuate the strain was made. The aim was to take normal plants at random and note the outcome. Nevertheless a great deal of natural selection has taken place. All abnormalities which interfere with or markedly reduce reproductive ability have been automatically eliminated. In this way many chlorophyll deficiencies, endosperm abnormalities and inherited sterility in tassels and ears, unfavorable conditions almost always present in every cross-pollinated

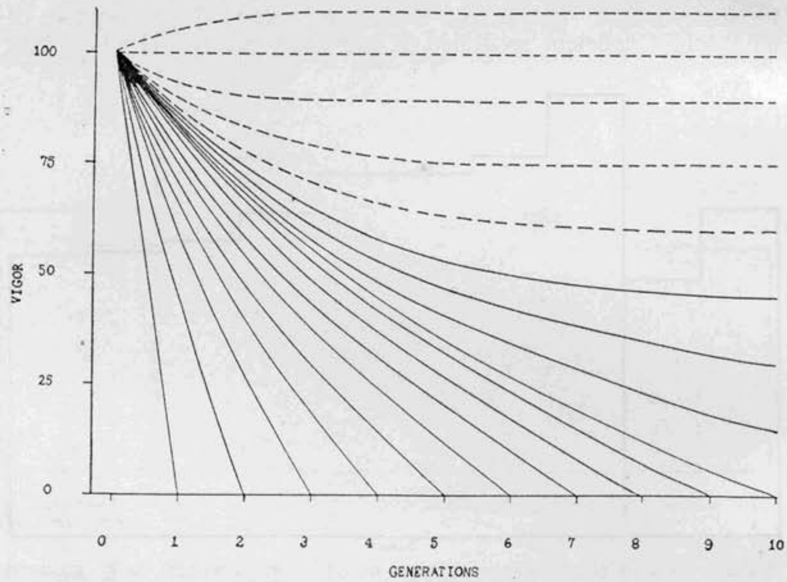

Figure 34. A diagrammatic representation of the actual and theoretical results of inbreeding corn. The solid lines represent strains which have already been obtained, the dotted lines those which may be expected when corn is worked with more extensively.

variety of corn, have been cleaned out. But this outcome of inbreeding, valuable as it may be, is less important than the control over the heredity made possible by hand pollination and the resulting fixity of type.

In common practice, selection with nearly all cross-fertilized plants has been based on the appearances of the plant or upon the performance of the progeny, and no adequate control of the heredity brought in from the pollen parent has been possible. As generally practised, corn breeding has been similar to a system of animal breeding in which selection is carried on only with the dams paying no attention whatever to the sires. The disastrous result that such a system would have upon purebred live-stock can readily be appreciated. With all cross-fertilized plants it would be theoretically possible to follow the method now used in animal breeding. Certain desirable individuals could be chosen as seed parents and others as pollen parents. Pollination could be made by hand and the progenies compared on the basis of their performance. There is no doubt that this system followed up as carefully as it is in mating farm animals would give equal results. But such a method is wholly impracticable on account of the small value of the individual plant. The time spent on selecting the

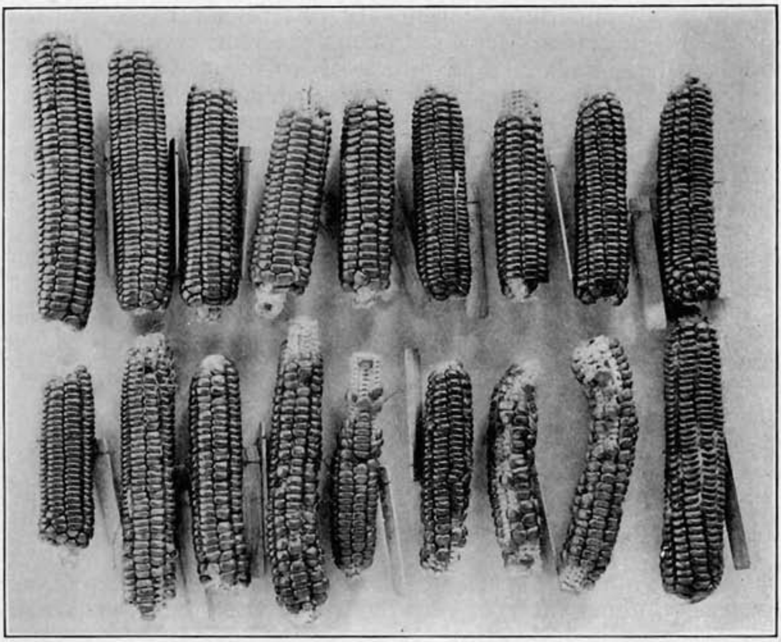

Figure 35. Self-pollinated ears grown on selected plants of Burwell's Yellow Flint, No. 40. Each ear is the starting point of a selected line. These are numbered 1 to 9, top row, and 10 to 18, bottom row, left to right.

parents and on pollinating each generation would not be repaid by the possible gains. Furthermore, with corn, selection is greatly handicapped due to the fact that the principal objective, production of grain, is not visible until after pollination.

A new method of attack, which will make possible a control of the heredity transmitted thru the pollen as well as thru the egg, is needed for all naturally cross-fertilized plants. Since inbreeding is a sorting-out process, selection carried on during the time the plants are being reduced to uniformity and constancy makes it possible to look for desirable qualities with a certainty of being able to hold them, when once secured, that has never before been possible. From this viewpoint inbreeding is not so important as a method of gaining the maximum effect of hybrid vigor when the inbred strains are crossed as it is of separating out and making visible the very best hereditary qualities that may exist in a heterozygous stock. Strains when once reduced to fixity remain the same indefinitely, barring mutations. With due regard to seasonal variation, crosses between inbred strains give the same result whenever the same combination is made. The uniform production of the first generation hybrids between homozygous strains is an important feature. In this respect cross-fertilized plants are equal to self-fertilized plants in uniformity and fixity of type and have the added advantage of crossing to bring together and use in the first generation the desirable qualities within the species, which in a self-fertilized organism can be used only when recombined and fixed in a homozygous condition. It should therefore be clearly understood that the crossing of inbred strains as such is without particular value and that the opportunity afforded to find and to fix the very best hereditary qualities possessed by a cross-bred race is the more important function of inbreeding. Crossing is merely a means of utilizing this good heredity by giving it maximum vigor. It is to be expected that many inbred strains will have only medium value and give no improvement over the original variety when crossed. The bulk of the germplasm in every population is mediocre. Of necessity only the exceptionally few will give outstanding results. For these reasons the outcome of selection in self-fertilized lines depends upon how extensively and skillfully it is applied.

Possibility of Obtaining Vigorous Inbred Strains.

Most of the inbred strains of corn so far produced have been reduced to about fifty per cent. or less of the production of the original cross-bred varieties. Some strains have failed to reproduce after one generation of self-fertilization. Others have persisted in a weakened condition for several generations and then perished. Still other strains are able to survive, but are continued only with the greatest difficulty. The majority of the self-fertilized lines, when uniformity and fixity of type are reached, are about one-third as productive as at the start. A few are exceptionally good. They grow more vigorously and yield more than the rest and are equally uniform and fixed in their type. But even the best of these are still below the original variety in amount or quality of grain produced. On the basis of hybrid vigor being due to dominance of the more favorable factors it is theoretically possible to secure inbred strains that will show little or no reduction in vigor, and a few may sometime be obtained that are even more vigorous and productive than the cross-bred variety. This is deduced from the fact that most heterozygous combinations of factors are less effective than the homozygous combinations of the same factors. Thus the cross of yellow and white corn gives a lighter color than pure yellow. The cross between a determinate growth type of tobacco with an indeterminate growth type (Jones, 1921) which involves a single factor, differs from either parent in size of plant and number of leaves. Dominance is seldom perfect and while there is little direct evidence in this respect for characters

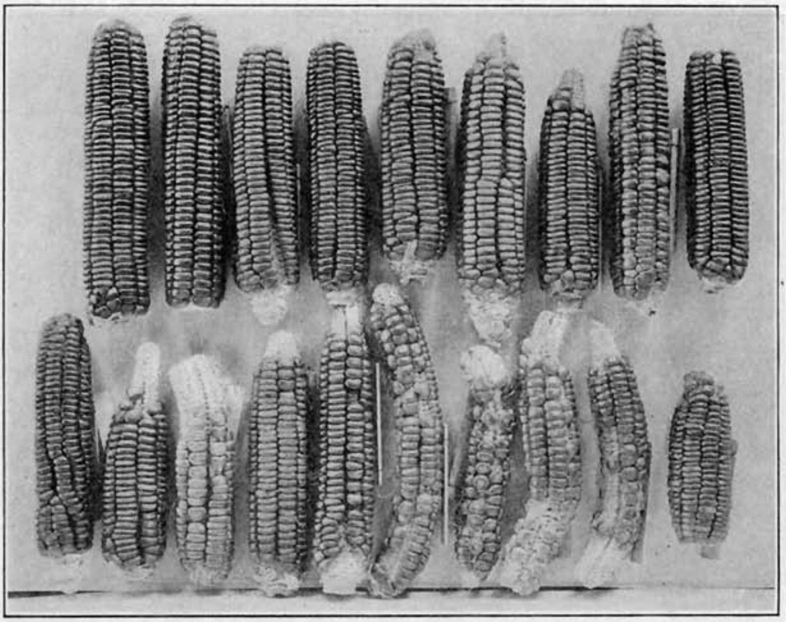

Figure 36. Self-pollinated ears grown on selected plants of Gold Nugget, No. 105. Each ear is the starting point of a selected line. These are numbered 2 to 10, top row, and 11 to 20 bottom row, left to right. (Ear 1 was shelled before photographing. It was similar to No. 2.)

which directly affect vigor there is every reason to expect that a homozygous combination of all the more favorable dominant growth factors will make possible a greater development than the heterozygous combinations of the same factors with weaker allelomorphs. However, as just noted, certain results are obtained from heterozygous combinations that can not be obtained from either factor alone. If there are many of these that play a part in growth vigor, then heterozygosity may be indispensable to maximum development. Moreover, recombinations of large number of factors are extremely difficult to obtain and since favorable and unfavorable growth factors are distributed indiscriminately throughout the hereditary mechanism the chances of securing self-fertilized strains of corn which equal the cross-bred varieties are so exceedingly small that there is little hope of obtaining them. The most that can reasonable be expected are inbred strains which are appreciably better than any that have so far been produced. The results that have already been obtained from self-fertilizing corn, and the theoretical possibilities, some of which may be attained in the future, are shown diagrammatically in figure 34.

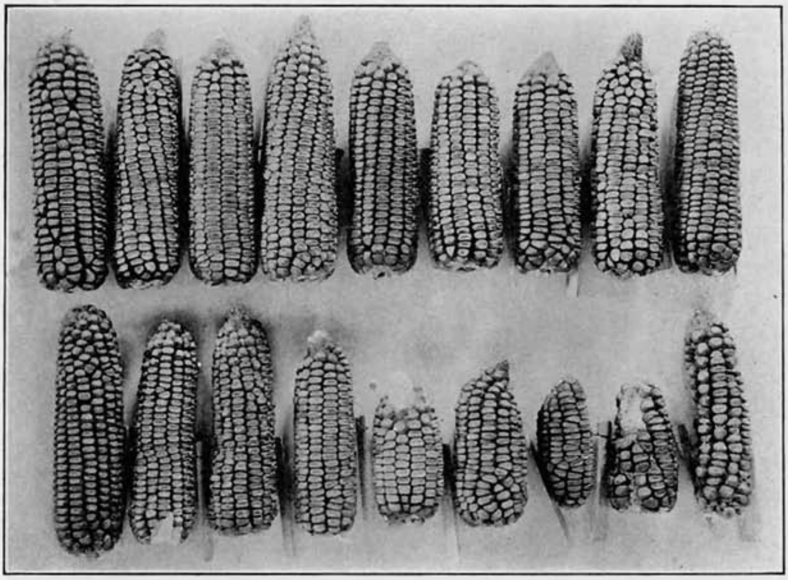

Figure 37. Self-pollinated ears grown on selected plants of Century Dent, No. 110. Each ear is the starting point of a selected line. These are numbered 1 to 9 top row and 10 to 18, bottom row, left to right.

Selection in Self-Fertilized Lines.

To demonstrate the value of inbreeding as a means of isolating good heredity a system of selection in self-fertilized lines was begun in 1918. Four varieties of corn were chosen as material with which to work. These varieties have been grown in Connecticut for many years and are well adapted. In a variety test of long duration they have proven to be among the best in production of grain and in other qualities. The four varieties are as follows:

Burwell's Yellow Flint, No. 30 and No. 40. An eight rowed yellow corn of the Canada Flint type. The ears are medium in size, one or two on the stalk. The plants are medium in maturity.

Gold Nugget, No. 105. An eight rowed yellow flint corn with large ears, broad kernels and heavy cobs. The stalks are large with few suckers. The plants mature late in the season.

Century Dent, No. 110. A light yellow dent corn with broad, smooth, shallow dented kernels. The ears are medium in size and have from 14 to 18 rows. The plants are medium in size and mature well in practically every season.

Beardsley's Leaming, No. 112. A yellow dent corn with tapering ears with 16 to 22 rows and small, shallow kernels. The stalks are large. This variety is later in maturing than Century Dent and is usually more productive.

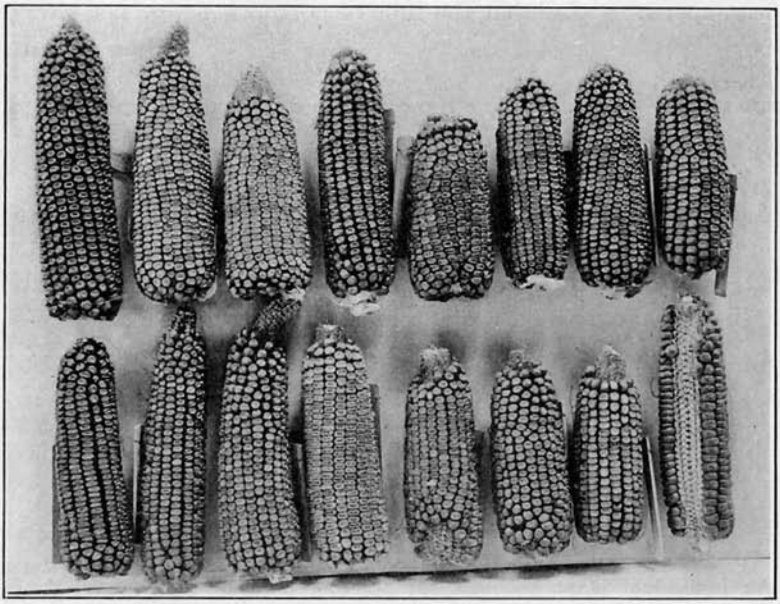

Figure 38. Self-pollinated ears grown on selected plants of Beardsley's Leaming, No. 112. Each ear is the starting point of a selected line. These are numbered 1 to 8, top row, and 9 to 16 bottom row.

The plan of procedure was to self-fertilize a number of the best plants in each of these four varieties and to use each of these plants as the starting point of an inbred line. These lines were to be continued by self-pollination of the best plants in each generation until uniformity and constancy were reached. Accordingly from about 60 plants each of the four varieties grown from a general mixed lot of seed, 20 plants of each variety were selected at pollinating time and self-fertilized. These four lots of ears are shown in figures 35 to 38. Some of the self-pollinated plants failed to set seed but all of the ears that had enough seed to work with were planted. The original hand-pollinated ears were ranked according to their appearance in size, form of the ear and quality of the seed. Ear number one represents the best, number two the next best and so on down. The ear numbers became the numbers for the self-fertilized lines derived from them. Therefore, the number of the line shows how its original progenitor was classified. It is of considerable interest to note to what extent good strains can be obtained from unpromising ears at the start.

Each self-pollinated ear was planted in a row the following year and five plants of each were again selected at pollinating time as the most desirable and were self-fertilized. It was noted that the best appearing plants at the time of pollination were not always

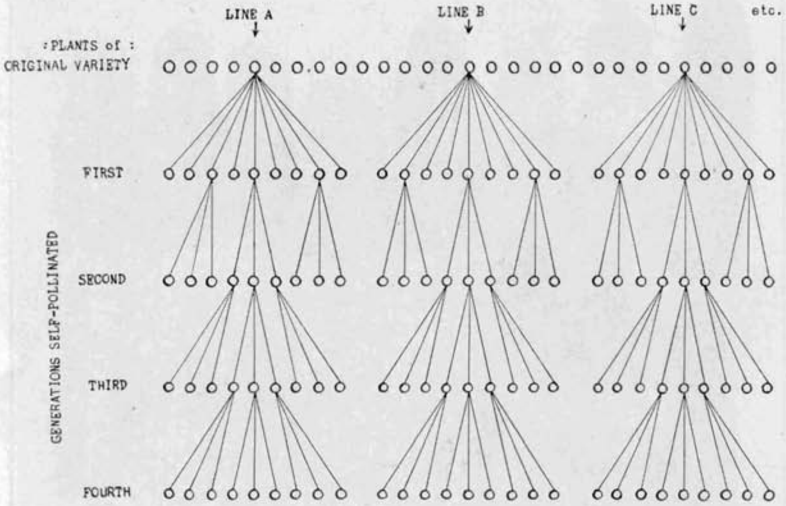

Figure 39. Diagram of a method of selection in self-fertilized lines. An individual plant becomes the starting point of each inbred strain. Three progenies are grown but only one is selected to continue the line.

the most productive at maturity. For this reason more plants were self-pollinated than there were progenies planted, thus allowing for some failures of pollination and also to permit of some selection among the hand pollinated ears. Also, in order to base selection upon progeny performance rather than upon the appearance of the seed ear, three progenies from each line were grown each year. At pollinating time the best appearing progeny was chosen and five plants were again self-fertilized, the other two progenies being discarded. This method of carrying on selection is shown diagrammatically in figure 39.

About thirty plants were grown in each progeny. From three to five times this number of seeds was planted and the poorest seedlings pulled out after they were well started, leaving the tallest and most vigorous plants. An even stand was obtained in most cases. The end plants in each row were usually avoided in selecting the plants for hand-pollination as these are nearly always larger and better developed than the others on account of their better opportunity to grow.

METHOD OF POLLINATION.

The plants were pollinated by hand as shown in figures 40 and 41. The general method used is as follows:* A three pound manila grocer's bag is placed over the ear shoot before the silks appear. The tassels are covered with an eight or ten pound bag as soon as they are above the upper leaves. When the silks are about three-fourths out, pollen is dusted over them and the tassel bag placed over the ear. Care is taken not to touch the silks or the inside of the tassel bags with the hands in order to avoid contamination with foreign pollen. If the silks extend more than three or four inches beyond the tip of the ear they are cut back with a knife sterilized in alcohol. After the first generation or two, out-crossed plants can be easily noted by their much greater size and darker green color so that contaminating pollen is not a cause for great concern. Effort is made to pollinate as rapidly as possible. Only one application of pollen is made. If sufficient seed does not result from this application the ears are not used. Some good plants are lost because all the pollen has been shed and has lost its viability before any silks appear. This tendency to protandry is accentuated in some inbred lines. Such strains could be maintained by sib-crossing but since this method of inbreeding is much less effective than self-fertilization in bringing about homozygosity the latter system has been rigidly adhered to. In this way sterility and recessive abnormalities of all kinds are most quickly eliminated.

SELECTION OF EARS FOR PLANTING.

Each hand-pollinated plant is tagged with a printed form upon which notes as to the character of the plants in the field and the hand-pollinated ears when mature are entered as follows:

Pedigree number
Field plot number
Height to ear-bearing node
Height to first branch on tassel
Number of ears containing seed
Number of leaves
Number of tillers
Posture, whether erect, leaning, bent, broken or fallen
Color and markings of foliage
Infection on plant
Smut on ear
Mold on ear
Number of rows of grain on ear, regularity of rows, and length of ear
Color and general character of seeds
Color and shape of cob.

* A method of pollinating proposed by Jenkins and known as the "bottle method" was also tried. Under our conditions it did not prove as satisfactory as the procedure described here.

At harvest these tags are transferred to the hand-pollinated ears. In choosing the three ears for planting in each line, from the five ears pollinated, the characters of the plants in the field as well as the size and appearance of the ears are taken into consideration, chief attention being given to ability to stand erect, color of foliage, freedom from smut and other infection on the plant and ear and absence of mold on the ears.

ELIMINATION OF SELF-FERTILIZED LINES.

In all, 86 self-fertilized lines were started, distributed among

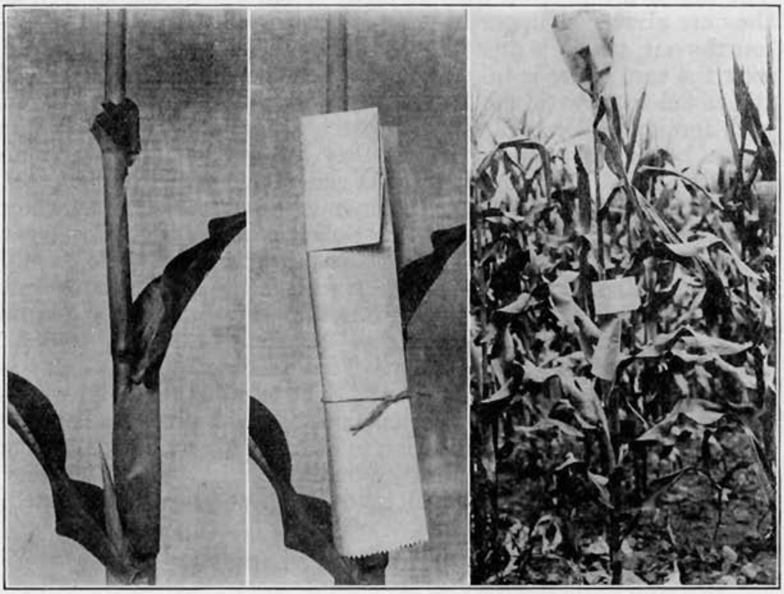

Figure 40. Plant bagged for hand pollination. Small bags can be used over the ear shoot and the tassel bag placed on the ear when pollinated. Wire clips are now used to hold the bags on the ear and tassel.

the four varieties as follows: From Burwell's Yellow Flint number 40 there were 18 ears self-pollinated in 1918, ranked and numbered from 1 to 18 in order of their excellence as shown in figure 35. In addition to these there were 14 ears of the same variety which had been self-fertilized in 1914 for another purpose and not used. These were included among the Burwell strains with the variety number 30 to distinguish them from the other strains which were ranked according to their appearance. The fact that these ears had been held five years before planting has interest in connection with the possible elimination of abnormalties due to the age of the seed, as will be noted later. From the Gold Nugget variety, number 105, twenty lines were started (figure 36); from Century Dent, number 110, eighteen lines (figure 37), and from Beardsley's Leaming, number 112, sixteen lines were started (figure 38).

The once self-pollinated ears beginning these 86 lines were planted in 1919 and hand-pollinated ears were obtained from all lines except one in Gold Nugget and two in Century Dent. These failures to produce seed in all five pollinations in each line may have been due to delayed pollination and unfavorable weather conditions. But since good ears were obtained in the other lines

Figure 41. Pollinating corn. Only one man is necessary for this operation. Care is taken not to touch the silks or the inside of tassel bag. If the silks are more than three inches long they are cut back to about one inch with a knife sterilized in alcohol.

it is fair to assume that these lines were less vigorous or for some reason were not as able to reproduce under this method of pollination. In the second generation two more lines were lost because no self-pollinated seed was obtained. In the third generation four lines were discontinued. In two of these no hand-pollinated ears were obtained, and the other two were so badly damaged by mold that they were discarded.

In the fourth generation eleven lines were eliminated. Nine were discarded because they were so very poor and unpromising that it was thought advisable not to carry them further. Some of these failed to produce any seed on any plants. All of the hand-pollinated ears of two lines proved to be out-crossed, due possibly to the fact that the bags covering the ears of the previous generation were broken and allowed foreign pollen to enter. By the fifth generation practically all of the lines had become uniform and stable. All that had survived up to this point gave promise of being able to continue indefinitely if sufficient effort was put forth and provided the season was not too unfavorable. During the course of the five-year selection period the following lines were eliminated for various reasons:

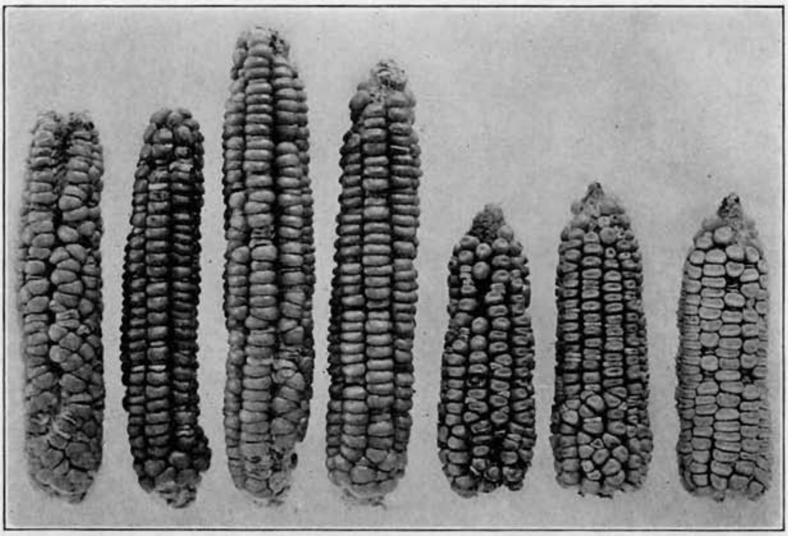

Figure 42. Self-fertilized ears showing defective or aborted seeds.

In No. 30, line 2 was accidentally lost.
In No. 40, lines 2, 5, 11, 12, 17, 18 were discarded.
In No. 105, lines 1, 4, 5, 12, 19 were lost or discarded.
In No. 110, lines 8, 12, 13, 14 were lost or discarded.
In No. 112, lines 2, 5, 11, 13 were discarded.

In all, 20 lines were not continued to the end of the fifth generation. Three of these were accidentally lost thru no fault of their own. The others were too poor to be carried along. An examination of the original ears from which these lines came (figures 35 to 38) shows no marked relation between their poor behavior and their appearance when first pollinated. Dividing each lot of ears into two equal groups and not counting the three lines that were accidentally lost, we find that seven from the best appearing lines at the start were discarded and ten from the poorest.

The original plan was to keep all lines that could be successfully propagated even though they became extremely poor. It was fully appreciated that inbred strains may themselves be very

Figure 43. Seedlings lacking chlorophyll are common hereditary variations in corn.

undesirable and still have potentially great value when crossed with other strains. For this reason no lines were discarded unless the amount of seed produced was so small that enough plants to permit satisfactory measurements could not be grown. Many lines were continued which were extremely weak, unproductive and showed markedly undesirable characters. They were continued to compare them in crossing with other strains. The results of these comparisons will be reported in a later publication. It should be emphasized here that the 20 lines, or 23 per cent. of the original number, which were lost or discarded, represent for the most part extremely poor and undesirable material that would probably be lost in any selection experiment. By growing a larger number of plants in order to give a greater opportunity for selection and by hand-pollinating a larger number of individuals it would probably have been possible to continue many of these lines and some might even have turned out to be good strains in the end. Whether it is worth while to work more intensively with a few lines or expend the same amount of time on a larger number of strains less intensively selected is one of the most important problems to be considered.

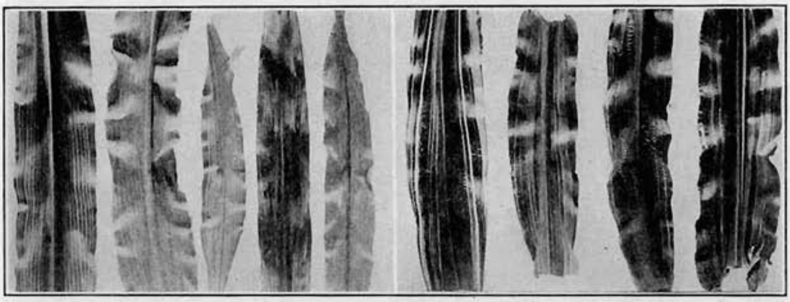

Figure. 44 Various types of chlorophyll deficiencies found in inbred strains of corn.

THE PRODUCTION OF ABNORMALITIES

An examination of the original ears after the first self-fertilization (figures 35 to 38) showed eight that were segregating for small, dull colored seeds that were clearly abnormal. These recessive seeds varied on different ears from almost entirely empty pericarps to seeds nearly normal in size but shriveled and opaque in appearance, as shown in figure 42. These aborted seeds, in most cases, failed to grow and those which did germinate, produced abnormal seedlings none of which reached maturity. The normal seeds from ears showing defectives when planted produced segregating ears on some of the plants in the following generation. In addition, five ears which were not clearly segregating in the first generation produced some ears with abnormal seeds in their second generation progenies. It has since been found that this defective seed condition is due to a large number of lethal or semi-lethal factors which are hereditarily distinct. They are widely distributed in all kinds of corn. In cross-pollinated plants only a few of these abortive

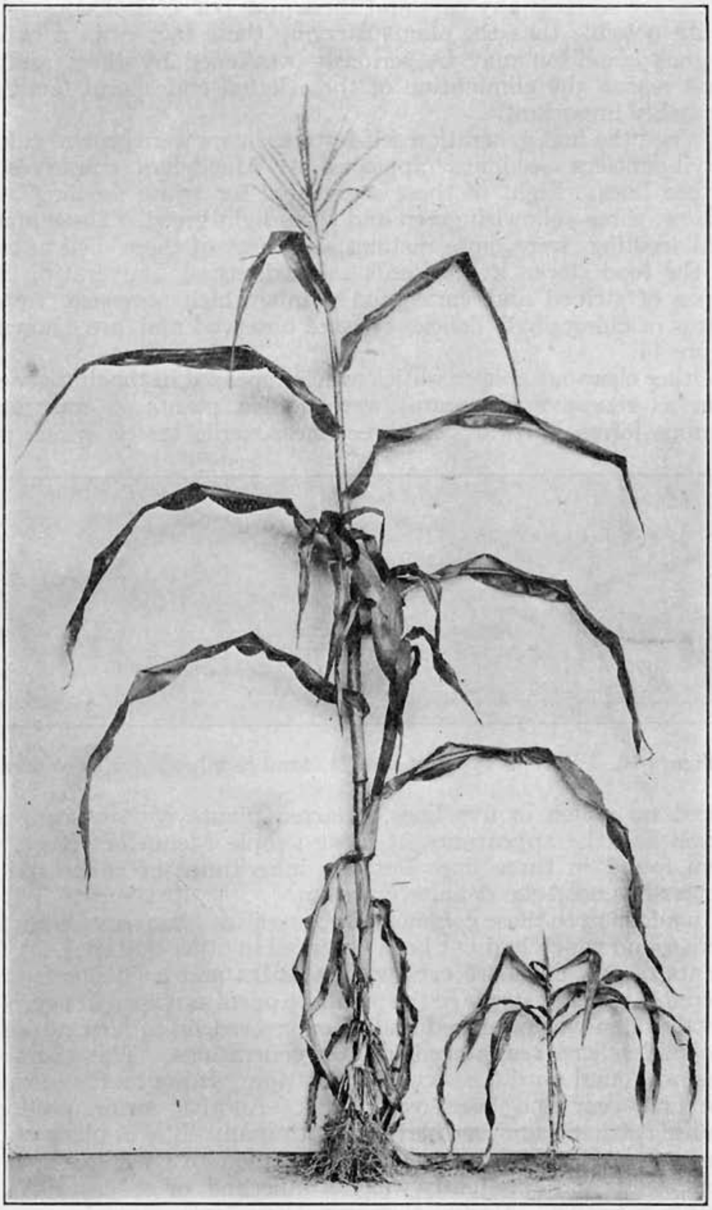

Figure 45. A chlorophyll-deficient dwarf compared to a normal plant in the same family.

seeds are seen on any ears and these are not conspicuous. It is quite possible that the plants carrying these factors in a heterozygous condition may be seriously weakened by them and for that reason the elimination of these lethal endosperm factors is probably important.

When the first generation self-fertilized ears were grown, chlorophyll-deficient seedlings appeared as Mendelian recessives in fifteen lines. Eight of these segregated for white seedlings, one yellow, three yellowish green and three light green. These abnormal seedlings were quite distinct and most of them died as soon as the food stored in the seeds was exhausted. Several distinct types of striped and variegated plants which represent various forms of chlorophyll deficiency were observed and are shown in figure 44.

Other clear-cut abnormalities which appeared in the first generation as recessive segregates were golden plants in four lines, various forms of dwarfs in three lines, sterile tassels which produced no pollen in five lines. Barren plants without ears and which had the appearance of being simple Mendelian recessives were found in three lines but the inheritance of such sterility factors has not been definitely proven.

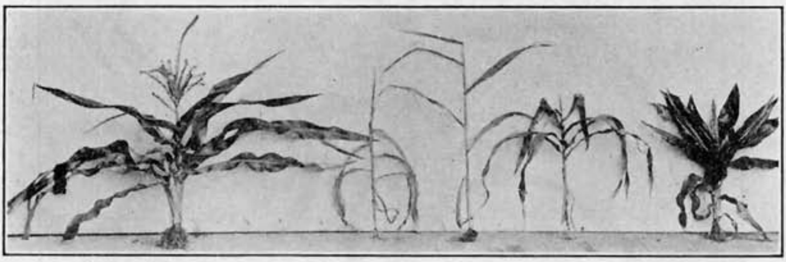

Figure 46. Various types of dwarfs found in inbred strains of corn.

In addition to these common abnormalities some new characters were found which had not been observed in other material. A few plants of one line bore ears with no silks and such plants were therefore entirely sterile in the pistillate parts as shown in figure 47. Good pollen was produced and when crossed on to normal plants the silkless ears reappeared in later generations. This character was not found until the second generation. It may have occurred the first year and been overlooked. Another strain produced square cobs and another had ears with many silks in place of one for each seed. This latter character failed to reappear in later generations and apparently was not inherited, or at least not as a simple recessive. Many other variations from normal occurred. They differed in degree of abnormality, some affecting the plants much more seriously than others.

In twelve lines no abnormalities were noted in the first two generations, but in the third or fourth generation, various types appeared, in the form of chlorophyll-deficient seedlings, striped and variegated plants, dwarfs, seedlings with tube leaves instead of normally flat, and plants with only the mid-ribs in place of normal leaves. In some of these cases recessive segregates may not have appeared in the first generations on account of elimination due to poor germination or they may have been thinned out with

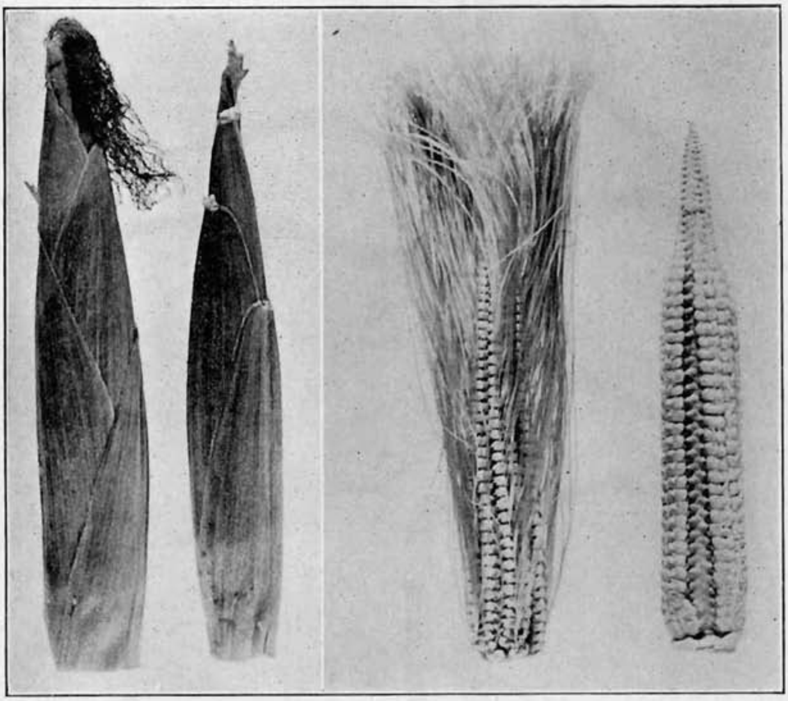

Figure 47. Silkless ears compared to normal specimens from the same family.

the weaker seedlings. In some cases, however, there seems to be no question that they are due either to original mutations or to delayed segregation resulting from some complicated mode of inheritance. A good illustration can be given in the production of the narrow-leaved plants shown in figure 48. Such a striking variation as this could not be easily overlooked. All the selected lines were carefully examined for abnormalities throughout the season, beginning with the early seedling stage. Narrow-leafed plants were first observed in the third generation in lines 112-13

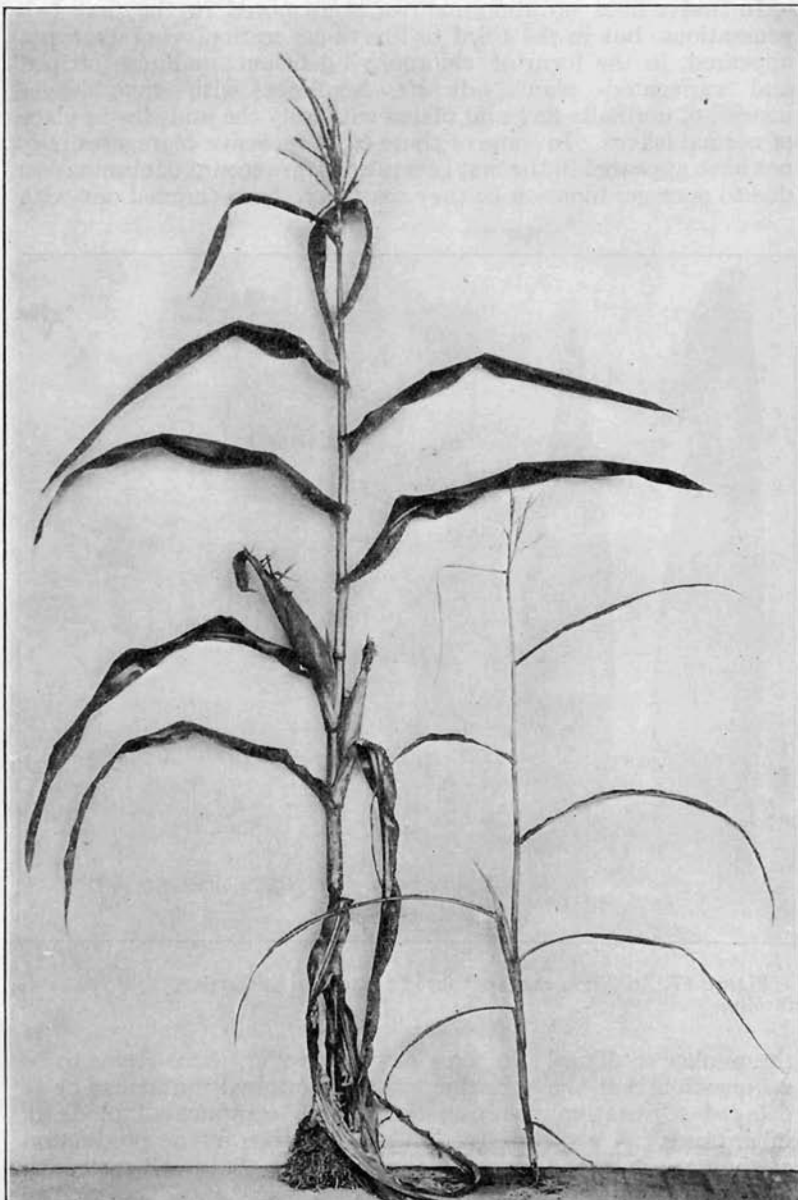

Figure 48. Plants with narrow leaves occurred in two inbred lines.

and 112-14. All three progenies of 112-13 produced some abnormal plants; two, nine and eleven narrow-leafed individuals appearing in the different progenies in a total of about 25 plants in each. This line had been segregating previously for dwarfs, golden plants, yellowish seedlings and striped dwarfs. Line 112-14 produced one narrow-leafed plant in the third generation. Though only normal plants were self-pollinated in the third generation, all of the fourth generation plants in line 112-13 were abnormal, being short and with streaked and wrinkled leaves varying in width from a mere mid-rib to nearly full width. The plants were so poor that no self-pollinated ears were obtained and the line was lost. Line 112-14 produced no narrow leaves in the fourth generation. All the plants were described as uniform, leafy but short in stature. In the fifth generation three progenies, all from ears borne on normal plants in the fourth generation, were grown. No plants were obtained from one and only a few in the other two. All of these had typical narrow leaves and were badly stunted. They made a feeble growth and produced no ears.

Pollen from typical narrow-leafed plants of the third generation out-crossed on to normal plants failed to show any abnormal plants in either the first or the second generation. Five self-fertilized progenies of the third generation were grown and in about 30 plants one narrow-leafed plant was found. The inheritance of this abnormality is not understood.

In the fourteen lines of Burwell's Flint which came from ears self-pollinated in 1914 and not planted until 1919 no abnormalities of any kind were noted in the first two generations. In the third and fourth a few chlorophyll-deficient seedlings, striped plants and tube leaves appeared. In contrast to this are the 18 lines of the same variety self-pollinated in 1918 and planted the following year which segregated the first generation for defective seeds, dwarf plants and chlorophyll-deficient seedlings in five lines. Five other lines of this lot were so poor they were discarded, while none of the 1914 lot were eliminated. Though the number of lines is too few to be conclusive it seems that the delay of five years in planting may have eliminated many abnormalities by the death of the seeds carrying them. A germination test of these ears, made in 1919, showed a viability ranging from 10 to 100 per cent. Eight of the 14 ears germinated 90 per cent. or less. None of the one year old self-pollinated ears of the same variety germinated less than 85 per cent. and only two were less than 95 per cent. There was clearly an elimination of seeds in the five-year resting period and this could easily have been selective, the seeds carrying the recessive abnormalities being less viable. If this is proven to be the case, some method of destroying the less viable seeds such as exposure to high temperature, alternate germinating and drying or similar harsh treatment may be an effective means of weeding out defective germplasm.

Many of these recessive abnormalities after they once appeared,

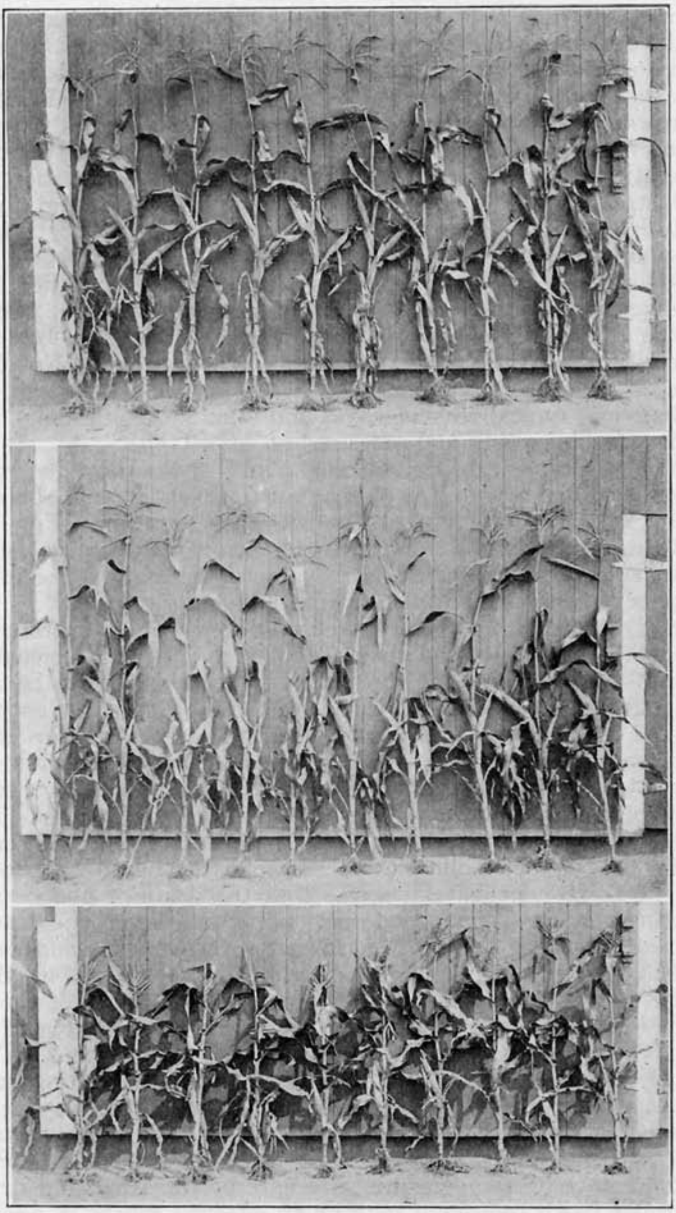

Figure 49. Representative plants of three flint lines; from top to bottom they are 40-4, 105-10, and 105-20.

kept reappearing in the following generations, but were finally eliminated, in every case except one, by the fifth generation. One line which was vigorous and productive and quite uniform in the fifth generation has segregated for white seedlings in every generation. Selection of progenies has usually been based upon productiveness and general appearances of the plants without regard to whether they were segregating for abnormalities or not.

Out of the original 86 lines only 32 lines or 37 per cent. showed no clear-cut recessive abnormalities during the five generations they were self-fertilized. As stated before, 13 lines or 15 pei cent. segregated for defective seeds, and 15 lines or 17 per cent. for chlorophyll-deficient seedlings. Many of the lines had several types of abnormality. In a lot of 575 self-fertilized ears from six varieties of white flint corn in another selection experiment there were found 19 ears or a little more than 3 per cent. segregating for defective seeds. Of these, 441 were grown and 40 lines or 9 per cent. were found to be segregating for chlorophyll-deficient seedlings. Hutchison self-fertilized 2,110 ears from a large number of different varieties of corn commonly grown in various parts of the country and found 3 per cent. segregating for defective seeds and 36 per cent. for various seedling characters, of which the greater number were chlorophyll deficiencies.

The widespread occurrence of these recessive abnormalities is fully established. In normally cross-pollinated plants they are comparatively rare in appearance since they are present as recessives in the heterozygous condition. To what extent, if any, they reduce growth in the heterozygous condition has not been established. Lindstrom (1920) suggests that in eliminating these recessive abnormalities many desirable factors with which they are linked may also be taken out. Since these recessives are presumably scattered throughout the chromosomes many other factors both good and poor will be taken out with them.

It has been argued that the recessive abnormalities tend to be eliminated by natural selection except in those cases where they happen to be closely linked with exceptionally favorable growth factors, in which case they would be preserved, and in weeding them out the factors which promote growth would be lost with them. The only answer to such an argument is to see what the facts are. Twenty-five lines segregating for clear-cut abnormalities gave progenies in the following generation, some with and some without the recessives. The 25 progenies which still carried the recessives averaged 50.8 bushels per acre yield in comparison with 50.4 bushels for the 25 progenies grown in the adjoining rows, and from which the abnormalities had been eliminated. An equally good stand was obtained in each case, as an excess of seed was planted and the recessive abnormalities thinned out. The difference in yield in the two lots is not significant. If there are favorable growth factors in the segregating progenies which are not present

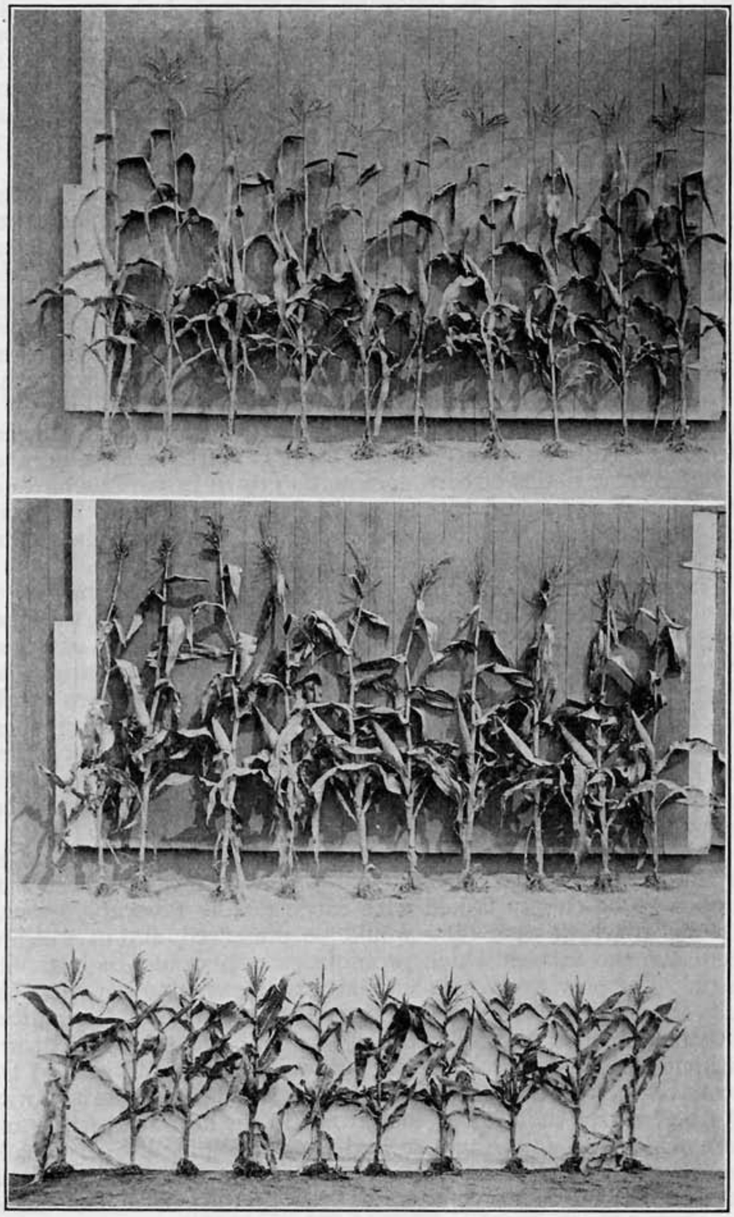

Figure 50. Representative plants of three early dent lines; from top to bottom they are 110-4, 6, 10.

in the non-segregating progenies from the same grand-parental plant they have no more effect than to counterbalance any weakening influence that the recessive abnormalities may have in the heterozygous condition.

Another comparison is made by finding the average per cent. reduction in yield of all segregating lines from the first generation to the fifth generation, by which time the abnormalities were eliminated. This reduction was found to be 57.1 per cent. compared to the reduction of 58.1 for all lines which were free from abnormalities at the start. If any favorable growth factors were lost when the recessive characters were weeded out, their departure caused no greater reduction in yield than took place in the other material from which no abnormalities were removed.

From this it seems evident that the chances are no greater for good factors to be eliminated than poor ones and with other things being equal it seems highly desirable to take out these clear-cut recessive abnormalities. In fact it is necessary, in most cases, to eliminate all lethal and semi-lethal factors, in order to bring the strains to uniformity.

THE APPROACH TO UNIFORMITY AND CONSTANCY.

As expected, the first and second generations were quite variable but in the third generation, after three successive self-fertilizations, a number of lines became fairly uniform in height of plant, color of foliage and in general characteristics. In the fourth generation the majority of the lines had become well fixed in their type, and after five generations all of the selected lines, with a few exceptions, were alike within themselves. This uniformity was apparent in the plants of each progeny and in the similarity among the several progenies of the same line. A few lines remained variable throughout the five generations. As a rule the lines that showed uniformity in the third generation declined somewhat in size and yield in the two subsequent generations. Practically all of the best strains can be picked in the fifth generation. Many of them can be recognized in the fourth and a few in the third. However, it is necessary to have a record of their performance during two and preferably three seasons after uniformity is reached in order to be sure that they are fixed in their type. Several strains that were considered to be very promising in the third generation declined so in vigor and productiveness in the two following generations that they were much inferior to strains that had, earlier, been far less promising. On the other hand a few of the most vigorous and productive lines in the fourth and fifth generations were not noted as being promising in the third. While it cannot be asserted positively that strains which are uniform and good in appearance during the fourth and fifth generations will maintain themselves without further reduction the evidence from the older inbreeding experiments indicates that

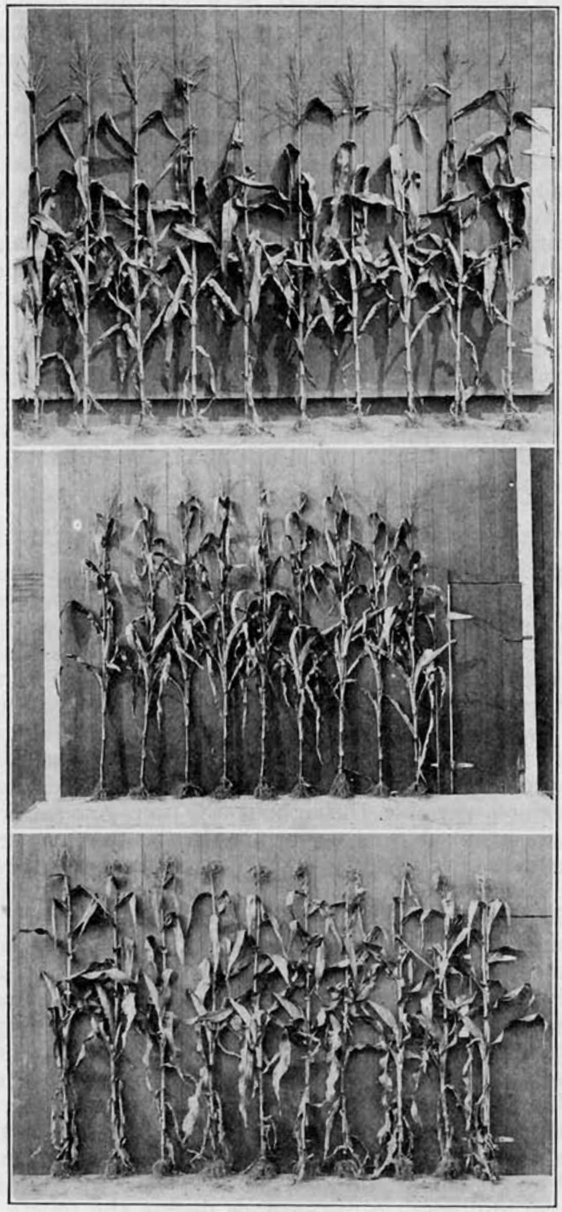

Figure 51. Representative plants of three late dent lines; from top to bottom they are: 112-1, 4, 9.

they can be expected to maintain their level of vigor without much loss. Therefore in carrying out a selection process of this kind the fourth and fifth generations are the most important in affording an opportunity to pick the best-appearing self-fertilized strains.

The selection process was carried out with the aim of securing the most vigorous and productive inbred strains, uniform and fixed in their type so that their good qualities could be maintained indefinitely. For this purpose five generations of self-fertilization are necessary in most cases.

Differences in the Selected Lines.

In the fourth generation all of the selected lines had become strikingly differentiated. Differences in height, color of foliage, size and shape of ears made each line distinct from every other line. In the Burwell Flint lines differences in average height ranged from 51 to 98 inches, in the Gold Nugget lines from 44 to 84, in the Century Dent from 44 to 76 and in the Beardsley's Leaming lines from 54 to 100. Color of foliage varied from very dark bluish green, through all gradations in shade to light green and yellowish green. In some lines the leaves were streaked with alternate rows of light and dark tissue. Various forms of fine and coarse flecking and mottling of the leaves were a regular feature of some strains while others were entirely free from this physiological irregularity of the chlorophyll.

The flint strains were most noticeably different in number of tillers. A number produced no large tillers and some had only a very few inconspicuous shoots from the base of the plants. Others branched very freely, producing many large branches on every plant. Many of these were as large as the main stalk and bore ears. Some strains regularly produced seeds in the tassels on nearly all plants while others never did this.

The ability to stand erect throughout the season is one feature that has been carefully selected for in all lines. Marked differences in this respect were shown, being greater in some seasons than in others. Certain lines regularly went down sometime during the latter part of the season while others stood stiffly erect up to maturity. Equally pronounced differences in time of flowering are shown by the lines derived from the same variety. Most of the lines matured satisfactorily every season while others were so late as to be barely able to ripen seed. The weakening effect of inbreeding delays maturity in all lines but in spite of this some were earlier in ripening than the variety from which they were derived. Along with these differences in maturity were great dissimilarities in character of the grain. The seeds of some were hard, translucent and bright colored; others were soft, dull colored and in some lines regularly moldy.

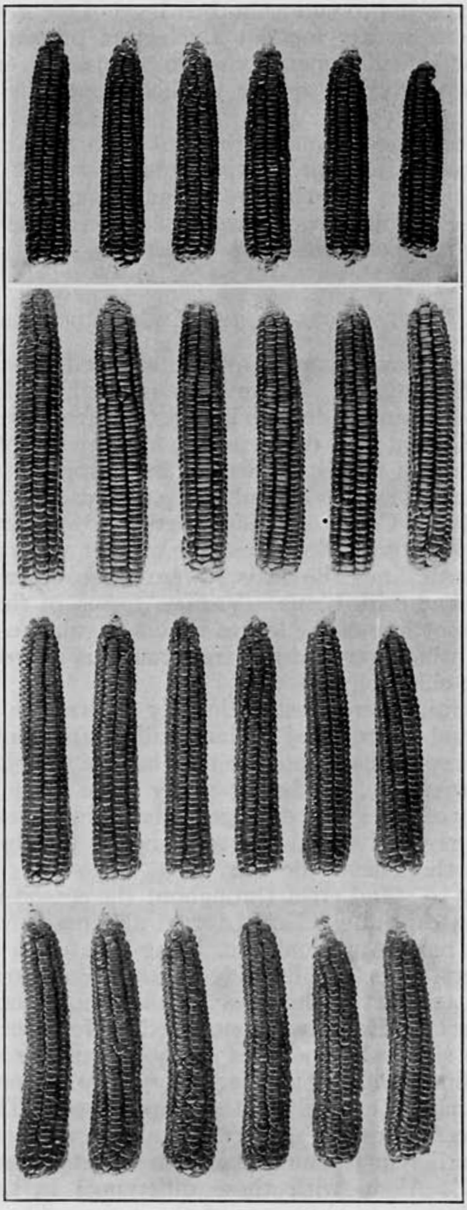

Figure 52. Representative ears of four productive Burwell Flint lines; from top to bottom they are: 30-19, 40-1, 7, 8.

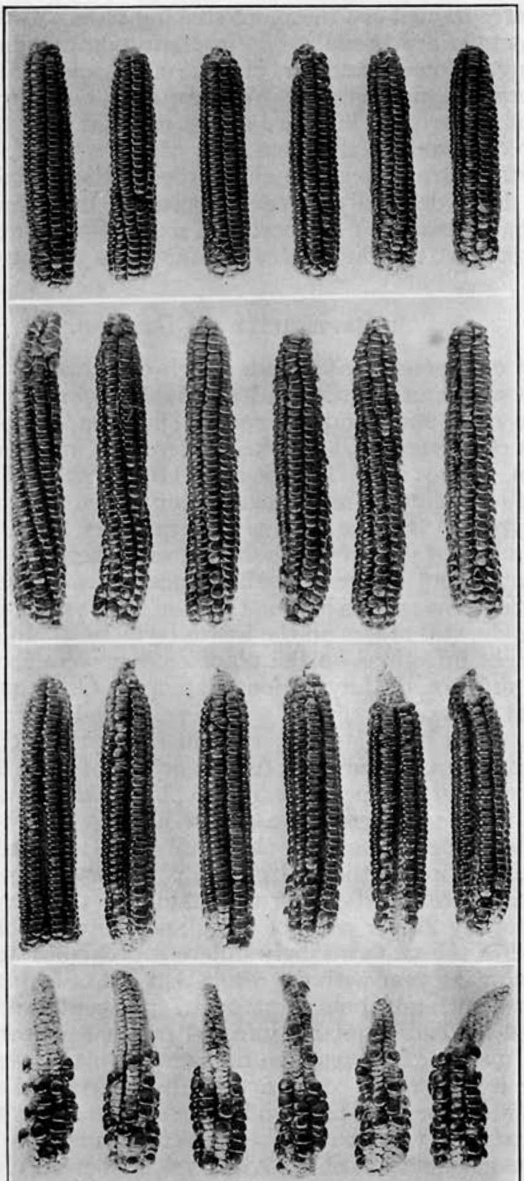

Figure 53. Representative ears of four unproductive Burwell Flint lines; from top to bottom they are: 30-5, 6, 40-15, 16.

The features named are the more striking ones. Differences in structural details are brought out in the accompanying illustrations showing the plants and ears of some of the selected lines in the fourth generation (figures 49 to 57). In details of structure and arrangement of parts the lines are so distinct that they can usually be easily recognized in the field and after harvest. In a few features certain strains may be alike. Some strains have similar plants but differ decidedly in ear structure. In others the ears are somewhat similar but are borne on markedly different plants. For the most part the differences are far more obvious than the similarities.

Susceptibility to Disease.

The most common diseases with which corn has to contend in Connecticut are smut (Ustilago), leaf blight (Helminthosporium), and various root, stalk and ear roots (Diplodea, Gibberella and other forms of Fusarium). Marked differences in smut infection were shown. Two lines 105-14 and 110-17 showed no smut infection on any plant in any progeny during the five generations they were grown. Eleven strains had no more than one plant affected in any one year throughout the same period. The place on the plant where the smut balls appeared was usually quite characteristic, some strains having them on the basal nodes, others at the ear node, still others on the leaves or tassels. In some lines numerous light infections on the plant or ears were shown which apparently did not do any serious damage. Other strains had many plants badly injured and sometimes killed outright during mid-season. The most striking case of segregation of susceptibility to parasitism by the smut fungus occured in line 110-3. In the first generation four per cent. of the plants were smutted. In the second three progenies were grown having twelve plants in each. In one progeny none of the plants had any indication of smut infection. In another all of the plants were smutted and most of them were killed during the middle of the summer. In the third progeny 27 per cent. of the plants were attacked. The original seed of the two strikingly different progenies was planted again the following year with the result that out of 57 plants of the resistant progeny, only one plant or 1.7 per cent. was infected. The smutted lot had 14 plants infected out of 31 grown, or 45.2 per cent. In the next generation no smutted plants were seen in the one line and 65.6 per cent. in the other. Marked differences were shown in the seeds of the two lines. Plants of the susceptible line were extremely weak but the seeds were normal in appearance. However the germination of these seeds was poor and in the fifth generation no plants were obtained. The resistant line produced more vigorous plants having a noticeably darker green color. All of the seeds produced on these plants were distinctly abnormal. When dry they were shriveled and discolored although not showing

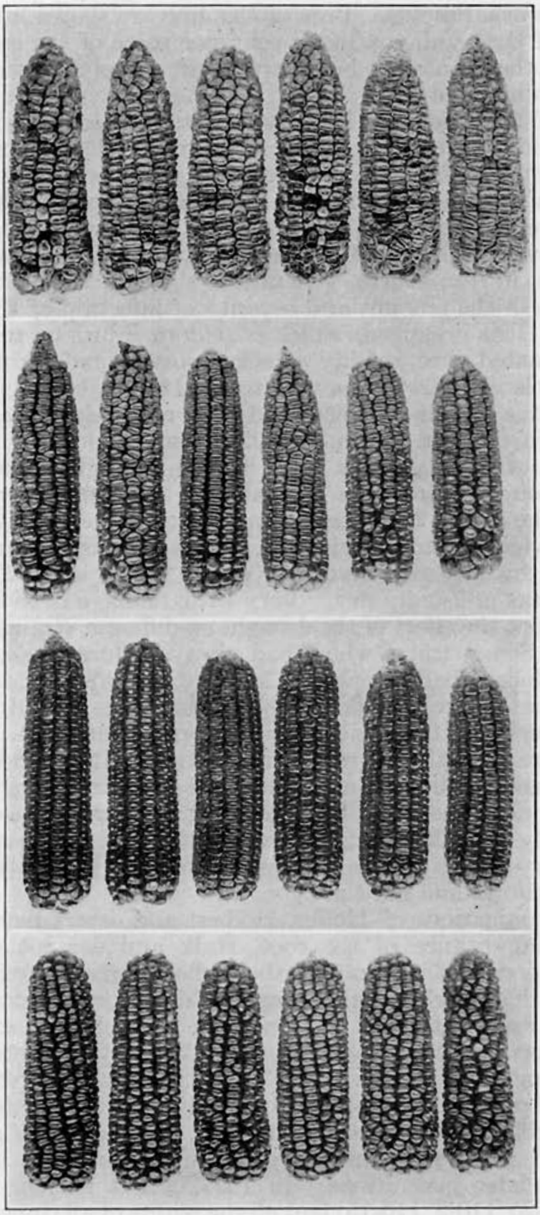

Figure 54. Representative ears of four Century Dent lines: from top to bottom they are: 110-3, 4, 5, 10.

any of the usual molds. Ears of this line are shown in figure 54. In spite of their unfavorable appearance some of the seeds germinate and the plants produced are about as good as the average inbred strain of the same variety.

None of the smut-free lines were outstandingly good in other respects and some of the most vigorous and productive strains now regularly show a high percentage of smut infection. The smut-free or low-smut strains may have value in crossing with other strains which have good qualities but are lacking in smut resistance.

The growing season of 1922 was unusually wet and the selected lines then in the fourth generation showed very pronounced differences in the amount and severity of infection of Helminthosporium. This organism, which is seldom injurious to ordinary cross-pollinated corn, readily attacks many inbred plants and on some completely kills the leaves after seed formation begins. Leaf blighting due to this organism had been noted each year in some lines but in the wet season of 1922 it was particularly injurious. Seventeen of the eighty-six lines showed heavy infection. Some of them lost all their foliage prematurely and the ears were badly stunted, the grains being small and poorly developed. Some of the most vigorous and productive strains in former years were so injured in this way as to give them a very low rating. The following year was unusually dry. Very little damage from this cause was seen, but the effect of the drought on different strains was very striking. Some strains which had always before produced green luxuriant foliage had their leaves killed at the sides and tips by the dry heat and were unproductive for that reason. Most of the strains which had been badly injured by leaf infection in the wet season were beautifully green throughout the dry period of 1923 and were among the best appearing and most promising of all the selected lines. These marked differences in different seasons makes it extremely difficult to judge the value of inbred strains and makes it necessary to test them during several years after they have become uniform and fixed in type.

The investigations of Hoffer, Holbert and others have emphasized the importance of the root, stalk and ear rot organisms attacking corn. The results of the earlier inbreeding experiments indicated that marked differences would be found among inbred plants to resist infection. Throughout the selection experiment great importance was placed on the ability of the plants to stand erect throughout the season and have the ears free from any indication of mold. Fallen plants or moldy ears were avoided whenever possible. The most outstanding differences in ability to stand erect and in freedom from mold on the ears, were seen in the third and later generations. In 1922, a wet season, four lines (30-6, 105-20, 110-2, 110-15) had all the plants of all three progenies erect throughout the season. This same year twelve lines (30-8, 30-9, 105-3, 105-18, 110-1, 110-2, 110-6, 110-7, 110-18, 112-6,

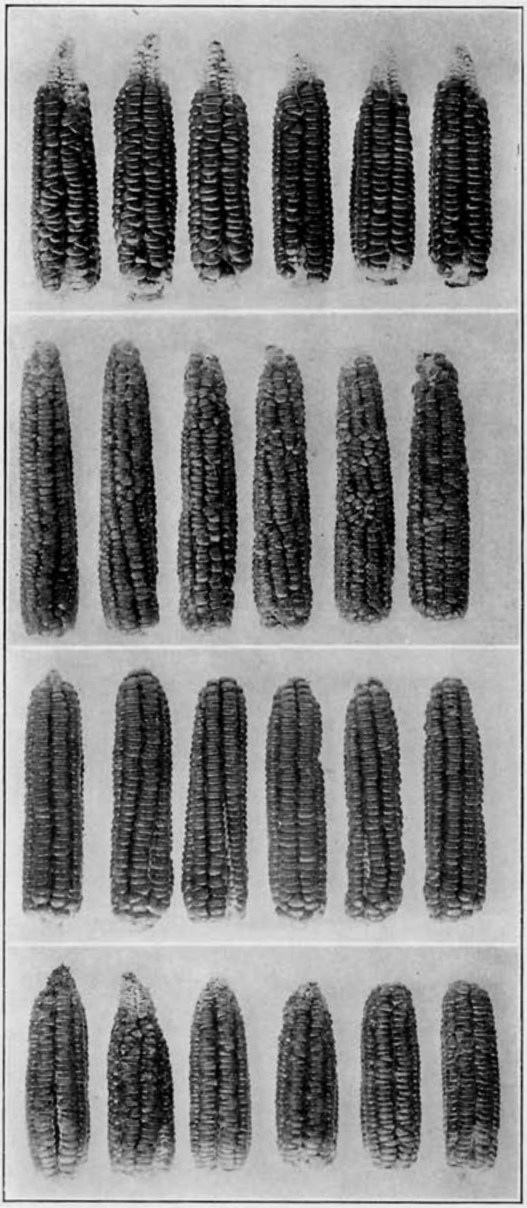

Figure 55. Representative ears of four Gold Nugget flint lines; from top to bottom they are: 105-3, 10, 17, 20.

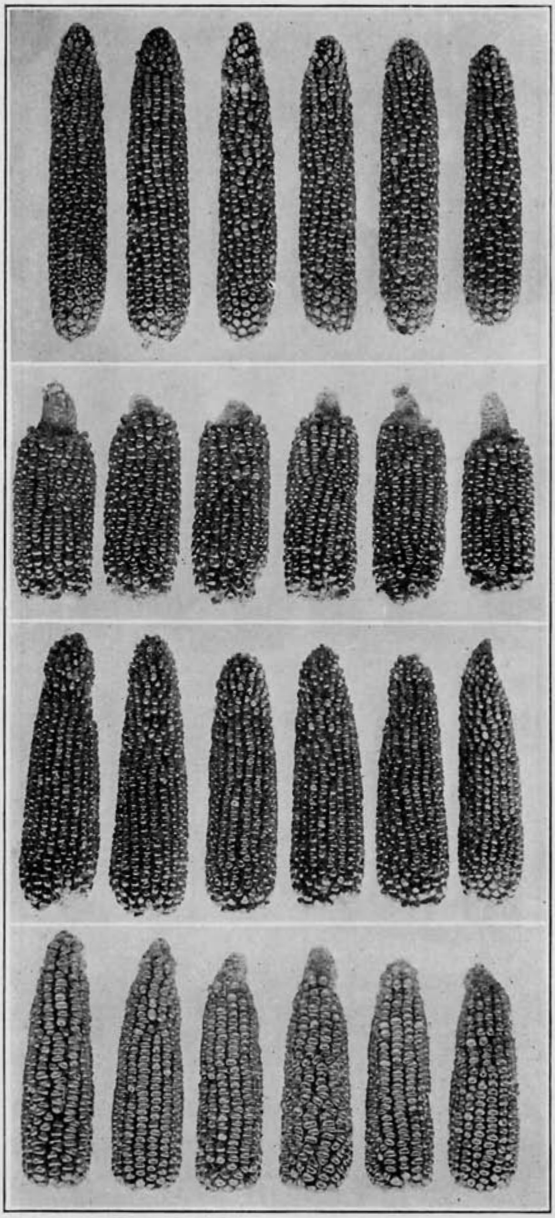

Figure 56. Representative ears of four productive Beardsley's Leaming lines; from top to bottom they are: 112-1, 4, 6, 9.

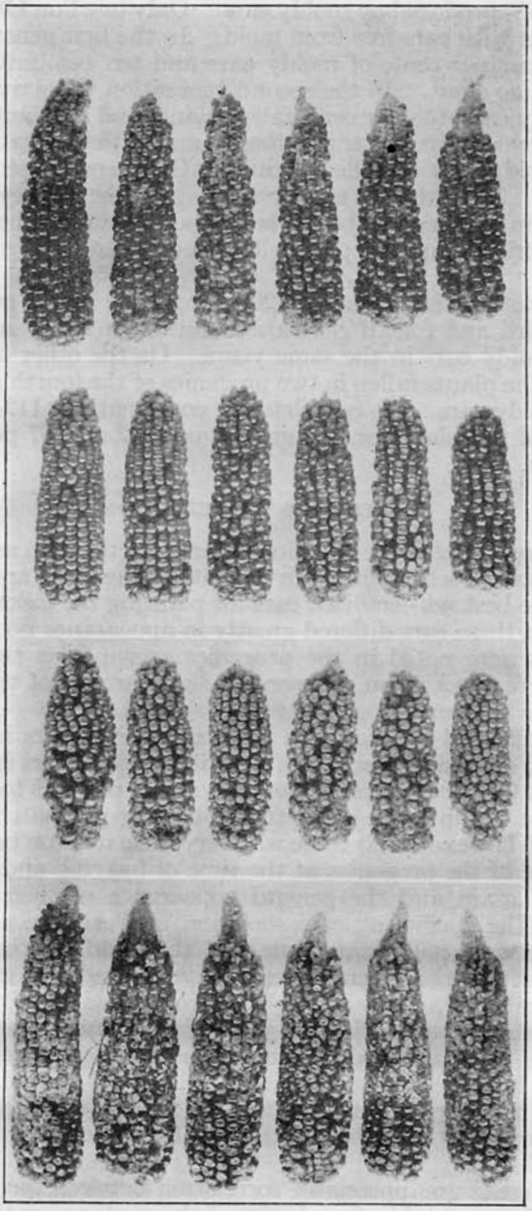

Figure 57. Representative ears of four unproductive Beardsley's Leaming lines; from top to bottom: 112-3, 10, 14, 15.

112-7, 112-8) produced no moldy ears. Only one line 110-2 had all plants erect with ears free from mold. In the first generation this line had four per cent. of moldy ears and ten per cent. of fallen plants but no smut. In the second generation there were ten per cent. moldy ears, ten per cent. fallen plants and no plants showing smut infection. In the third, fourth and fifth generations there was no mold, smut or fallen plants on the three progenies grown each year. This strain is also productive for the variety, although surpassed in this respect by several other strains. The seeds are hard and bright but very pale yellow in color and almost white on top.

In contrast to this is line 105-20 with all the plants erect in the second, third and fourth generations but with 29, 17 and 44 per cent. of moldy ears in the same years. On the other hand, 40-8 had all of the plants fallen in two progenies of the fourth generation and no moldy ears. To complete the combinations 112-11 had 87 per cent. of the plants on the ground in 1922 and 67 per cent. of ears moldy.

Criterions of Selection.

At the beginning of the selection experiment the plan as previously stated was to self-pollinate five plants in each line and to select three of the best self-fertilized ears for planting the following year. Even when these ears differed greatly in appearance no consistent differences were noted in the progenies grown from them. The coefficient of association between the appearance of the ear and yield of the different progenies within several lines is —.18. This indicates that self-pollinating a large number of ears in order to make more extensive selection of desirable looking ears is of doubtful value. Of the three progenies grown only one was to be chosen to continue the line, the other two not being pollinated. It was soon noted, however, that there was very little relation between the appearance of the progenies at the time of bagging and their production of grain and the general appearance of their plants at harvest. The coefficient of association between the appearance of the plants at pollinating time and the yield of the different progenies within the several lines is —.28. Seedlings were grown in the greenhouse and their weight and height after thirty days of growth were compared with the yield of the same progenies in the field. The third and fourth generations showed that those progenies that had the tallest seedlings yielded 1.6 bushels per acre more than the other progenies in the same lines. This difference is hardly enough to make a selection of the progenies on this basis worth while.

Since there is no appreciable correlation between the characters of the seed ear, weight of seed, size of the seedling, or the appearance of the plants at pollinating time and production of grain the only selection of progenies that can be made with any degree of effectiveness is at maturity. Here also yield is highly influenced by the amount of heterozygosity remaining. In some lines there are more homozygous combinations than in others and they are correspondingly less vigorous and productive although they may be potentially more desirable. For this reason final judgment must be left until the plants are reduced to uniformity and constancy. Hence it is interesting to note what resemblance the resulting inbred strains, when finally reduced to uniformity and fixity of type, have to the same strains in the first generations of inbreeding.

Classification of Selected Lines.

Taking into consideration all features of these selected lines as they grow in the field and after harvest in the fourth and fifth generations and giving most importance to the production of bright sound grain, the four outstanding good and poor strains in each variety are listed as follows, with their yields in bushels per acre in the fifth generation compared with that of the original variety grown the same year:

Burwell's Flint 51.2

Good Lines		*Poor Lines*	
Number	*Yield*	*Number*	*Yield*
30–5	12.2	30–10	44.2
30–19	15.3	30–18	18.3
40–4	33.6	40–3	35.1
40–8	25.9	40–16	24.4

Gold Nugget 54.0

Good Lines		*Poor Lines*	
Number	*Yield*	*Number*	*Yield*
105–11	29.0	105–3	9.2
105–15	33.6	105–8	13.7
105–17	22.9	105–13	7.6
105–20	10.7	105–16	29.0

Century Dent 48.3

Good Lines		*Poor Lines*	
Number	*Yield*	*Number*	*Yield*
110–2	15.3	110–1	28.9
110–4	16.8	110–9	4.6
110–5	10.7	110–15	1.5
110–10	19.8	110–17	12.2

Beardsley's Leaming 49.5

Good Lines		*Poor Lines*	
Number	*Yield*	*Number*	*Yield*
112–1	42.7	112–3	10.7
112–6	27.5	112–7	21.4
112–9	33.6	112–14	.0
112–12	12.2	112–16	9.2

This is purely an arbitrary classification based upon the general appearance of the plants and ears. Some of the poor lines yielded more than the good lines but produced a very poor quality of grain. The original ears from which these lines descended (figures 35 to 38) show that there is no relation between the good and poor strains after uniformity was attained and the appearance of the seed ears from which they came. Low and high numbers are represented about equally in the good and poor strains.

Correlation Between the First and Last Generations.

In order to find out whether the elimination of the poor lines at the beginning of the inbreeding period is advisable, the correlation

Table VIII.

Coefficients of association between early and later generations of self-fertilized corn.

Generations Compared Variety	1–4 Height	1–4 Mold	1–5 Tillers	2–5 Smut	1–4 Yield
Burwell's Flint	.60	.89	.64	—.08	0
Gold Nugget	.35	0	.38	.38	.14
Century Dent	.80	.80	—.72	.72	.28
Beardsley's Leaming	.95	.38	.50	.20	.50
Ave. Flints	.50	.65	.55	.10	.05
Ave. Dents	.89	.63	.17	.52	.38
Average	.71	.64	.27	.27	.19

between the behavior of the plants in the first generation and the last generation has been worked out for the most important characters. In Table VIII are shown the coefficients of association

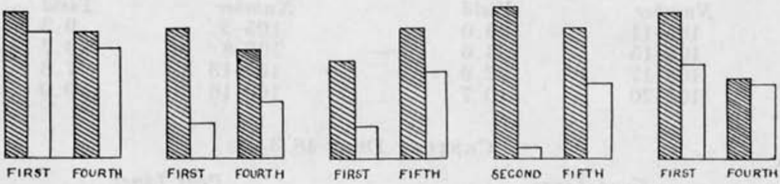

Figure 58. Diagram representing the average of the upper and lower groups in the first generations and the average of the same lines in the last generations, based on the data in Table IX.

between the first or second inbred generation and the fourth or fifth for height of plant, per cent. moldy ears, number of tillers, per cent smutted plants, and yield of grain. The fifth generation, grown in 1923, was so variable on account of the extremely dry season affecting different parts of the field unevenly that the coefficients for height and yield are based on the first and fourth generations. There was very little smut infection in the first generation and practically no mold in the fifth so that the coefficient for per cent. smut is based upon the second and fifth generations and for per cent. mold upon the first and fourth.

The figures show a fairly high association for height of plant and moldy ears. This means that by selecting the highest lines in the first generation the resulting inbred strains in the fourth generation would tend to be taller than the average. Similarly, by selecting lines at the start that were free from mold, inbred strains could

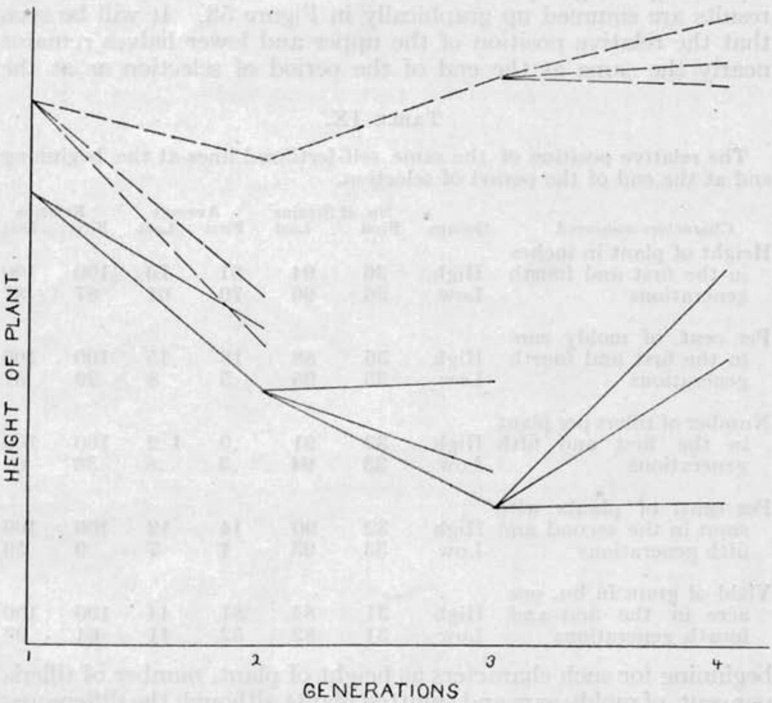

Figure 59. Graph showing the behavior of two lines with respect to height during four generations of self-fertilization, selected for vigor and productiveness but not for height. From one to three progenies are grown in each generation.

finally be attained that would on the average be freer from moldy ears than other strains which showed more mold at the start. This relation does not hold so well for the other characters; number of tillers and per cent. smut. For these the coefficients are low and in two of the varieties a negative correlation is shown. This means that lines without tillers and showing low smut infection may be obtained from plants at the start which have tillers and are susceptible to smut infection.

Another method of bringing out the relation between the several lines at the start and at the end of the selection period is to separate all the lines of each variety into the upper and lower halves, with respect to the characters studied, in the first generation and then compare the average of these two groups with the averages of the same lines after being inbred for four or five generations. This has been done in Table IX, making the separation within each variety into equal sized groups in the first generation. Thus the basis for separating the groups is the median instead of the mean. The results are summed up graphically in Figure 58. It will be seen that the relative position of the upper and lower halves remains nearly the same at the end of the period of selection as at the

TABLE IX.

The relative position of the same self-fertilized lines at the beginning and at the end of the period of selection.

Characters measured	Groups	No. of Strains First	No. of Strains Last	Average First	Average Last	Relative First	Relative Last
Height of plant in inches in the first and fourth generations	High	36	94	81	70	100	100
	Low	36	96	70	61	87	87
Per cent. of moldy ears in the first and fourth generations	High	36	88	18	15	100	100
	Low	35	95	5	8	29	57
Number of tillers per plant in the first and fifth generations	High	32	91	.9	1.2	100	100
	Low	33	94	.3	.8	36	67
Per cent. of plants with smut in the second and fifth generations	High	32	90	14	12	100	100
	Low	33	93	1	7	9	59
Yield of grain in bu. per acre in the first and fourth generations	High	31	84	81	44	100	100
	Low	31	82	52	41	64	93

beginning for such characters as height of plant, number of tillers, per cent. of moldy ears and smutted plants although the differences are generally less at the close than at the start. This tendency to change during the period of inbreeding is most marked for yield of grain. In this respect the high and low groups are very nearly alike at the end of the selection period in spite of the fact that all along attention has been given to productiveness. These results indicate that it is unwise to eliminate the unproductive strains in the first generations, as from them lines may be obtained that are as productive as those from high yielders at the start. Other characters can apparently be somewhat more surely selected for at the beginning of the inbreeding period. If such characters as freedom from mold and smut are of chief importance it might be advisable to eliminate those lines which show much mold and smut in the first inbred generations.

The general tendency for some of the lines to hold the same relative position throughout the process of selection is illustrated by the height of plant of two lines shown graphically in figure 59. In the first inbred generation the two lines averaged 69 and 77 inches in height. In the second generation two progenies overlapped but from then on they were clearly distinct, the difference in height increasing until the end of the selection period. The same result is shown in the average number of tillers per plant of two other lines as brought out in figure 60. Differing at the start the two lines remained distinctly different in all their progenies throughout the period of inbreeding. In marked contrast to this is the result shown graphically in figure 61. Two lines differing noticeably in their number of tillers changed positions so that in

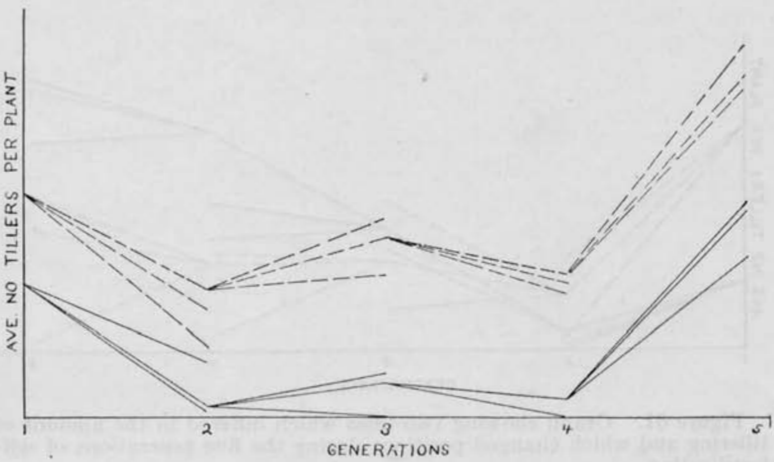

Figure 60. Graph showing the behavior of two lines with respect to tillering during five generations of self-fertilization, selected for vigor and productiveness but not for the number of tillers. The relative position of these two lines remained the same.

the end the few tiller strain at the start averaged more tillers on all progenies than did the many tiller strain. Similarly two strains which were alike in this respect at the start became extremely different as uniformity and constancy was reached, as shown in figure 62.

Limiting Factors.

In planning and carrying out a selection program the best procedure will depend upon the number of plants which can be grown and the number of hand pollinations which can be made in a season. Where the facilities available for artificial pollination is the limiting factor, and this is usually the case, the best procedure is to self-pollinate just enough plants to continue as many lines as possible until a reasonable degree of homozygosity is reached. If the amount of land available to grow the plants is the limiting factor it would be better to pollinate a larger number of plants within each line, although extensive selection within a progeny has been shown to have little value, as the better individuals are almost certain to be more heterozygous, making it difficult to arrive at their true value. More attention should be paid to increasing the number of progenies within the more desirable appearing lines, basing selection on their behavior throughout the season and their uniformity and productiveness at maturity.

The method now being used at this station is to grow three progenies in each line and to pollinate two plants in each progeny. On the basis of the general appearance of the plants in the field and

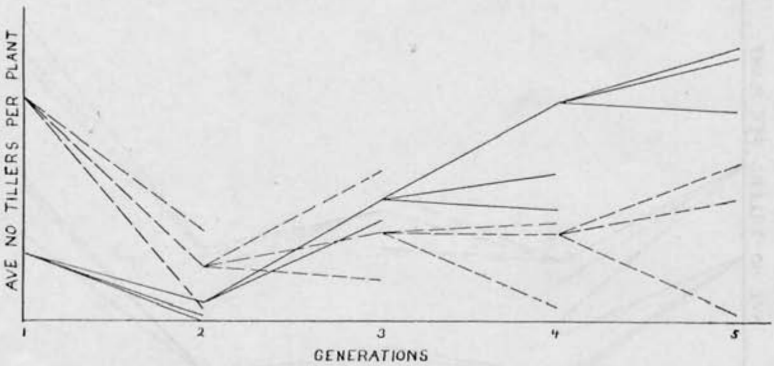

Figure 61. Graph showing two lines which differed in the amount of tillering and which changed positions during the five generations of self-fertilization.

their productiveness at maturity the best and second best progenies are noted where there is an appreciable difference. Two ears from the best progeny and one from the second best are used for planting the following year. If no differences are shown, one ear from each of the three is planted. This procedure is based upon the results in the five-year selection experiment described above in which no reliable criterions of selection were found which could be used before the time of pollination. It is still provisional and will be modified as future experience justifies. It is possible that better results can be obtained by paying still less attention to selection during the reduction period than the method outlined. By expending the same amount of time and effort on more lines, growing only one progeny in each generation and pollinating only enough plants to insure the perpetuation of the strain until uniformity and constancy are reached, more diverse material would be available from which to select the best inbred strains. In this procedure there would be the possibility, and even probability, of missing altogether valuable material which might exist in some lines. However, since it has been shown that many of the lines change greatly during the reduction process, selection during this period will always be somewhat ineffective. From a theoretical standpoint the best method is the one which will produce the largest number of fixed strains from which to choose the ones best suited to the purpose for which they are to be used.

In this connection one further point should be mentioned. Whenever any particularly outstandingly good strain has been obtained there is the possibility that still better material may exist in that strain in the earlier generations. This would indicate that it

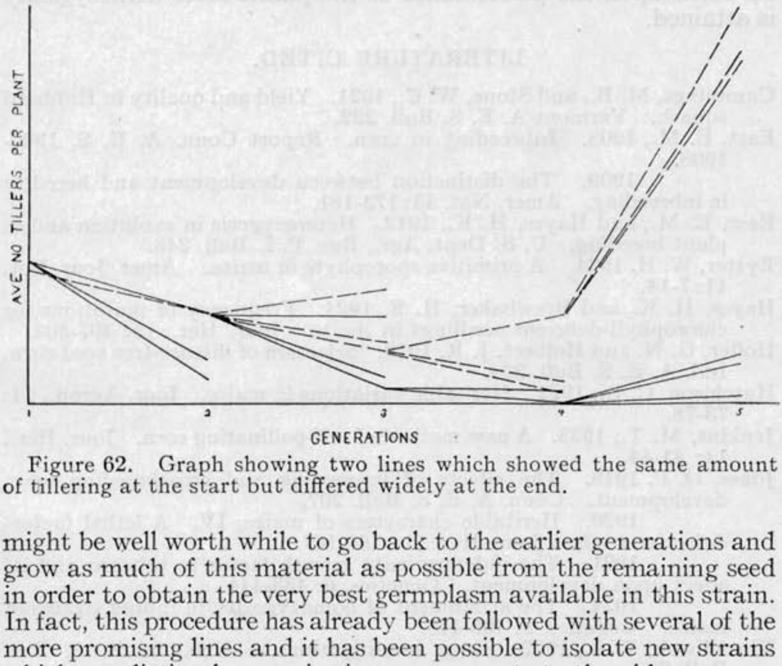

Figure 62. Graph showing two lines which showed the same amount of tillering at the start but differed widely at the end.

might be well worth while to go back to the earlier generations and grow as much of this material as possible from the remaining seed in order to obtain the very best germplasm available in this strain. In fact, this procedure has already been followed with several of the more promising lines and it has been possible to isolate new strains which are distinctly superior in some respects to the old ones.

CONCLUSION.

The one fact that stands out from the results secured in this selection experiment is that there is no single criterion by which high-yielding strains can be obtained. During the process of inbreeding, with the resulting segregation and recombination and the automatic elimination of heterozygous combinations of factors, selection for particular characters is somewhat effective. By choosing tall plants as progenitors in each generation tall strains can be produced. By selecting plants free from tillers, strains with few tillers can be obtained. Similarly, freedom from disease infection, as far as resistance is inherited, can be expected by selecting during the reduction period only those plants which show no infection in fields where infection is present. Even with these characters the association is far from complete. But productiveness, yield of grain, which sums up the plant's entire energies shows no such simple relation. High yielding strains may come, and have come, from plants which are poor producers. Promising strains during the first generations may be very unproductive or undesirable in some respect when finally reduced to uniformity and constancy. This emphasizes the fact that effective selection must be based upon the performance of the plants after homozygosity is attained.

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