CONTINUED INBREEDING IN MAIZE

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THE investigation of the effects of inbreeding maize begun by E. M. EAST in 1904 has been continued to the present time. The results of 30 generations of self-fertilization are here summarized. All surviving lines have been reduced to a high degree of uniformity and constancy. Variations of a degenerative nature have occurred from time to time and no doubt will continue to occur. Such variations have caused the extinction of at least one line and may prevent the continuation of some or all of the surviving lines. For the past ten generations however, they have been able to exist at about the same level of vigor without appreciable change.

These inbred lines were started from a number of self-pollinated plants of the Leaming variety grown at the Illinois Agricultural Experiment Station in 1904¹. This variety was known locally as Chester's Leaming and was selected as one of the most productive types of corn grown in that locality at that time. At least 12 plants were hand pollinated at the start but when the first detailed report on these inbreds was made in 1912 (EAST and HAYES) only four lines had been continued in Connecticut. These were numbers 1-6, 1-7, 1-9, and 1-12.

Number 1-12 was the least productive of the four, yielding only two bushels per acre in 1911 while the others gave 28, 25, and 26 respectively. This 1-12 line was either discontinued or lost sometime between 1911 and 1914. The 1-7 line was separated into two lines in the second generation. One of these, the 1-7-1-1 line, has been continued to the present time. The 1-7-1-2 line was lost in generation twenty for reasons that are not clearly understood. In about generation eighteen several plants showed a peculiar streaking of the leaves. Although seed was saved from only normal plants the condition increased until in generation twenty all of the plants were affected. No selfed seed was secured from a number of pollinations. Attempts to restore the line from old seed were unavailing.

The abnormality seemed to be in the production of chlorophyll. Affected plants were faintly striped or streaked with indistinct light green or yellowish green areas on the leaves. These plants produced pollen but rarely any seed. They were crossed on to normal plants in the same line and in unrelated lines. In both cases abnormal plants appeared in the first and later generations in varying numbers and with different degrees of abnormality.

¹ In 1912, EAST and HAYES stated that the first self-fertilized generations were grown in 1906 from hand-pollinated seed produced in 1905, but an earlier publication by EAST in 1909 presented results which show that the first self-pollinations must have been made in 1904.

In the self-fertilized line the abnormality increased steadily in the proportion of plants affected and the degree to which they were reduced in size and vigor until finally no seed was obtained. This variation has not appeared in any other line.

TABLE 1
The effect of thirty generations of self-fertilization upon the height of plant and yield of grain in maize.

NUMBER OF GENERATIONS SELFED	I-6		I-7		I-9	
	HEIGHT INCHES	YIELD BU. PER ACRE	HEIGHT INCHES	YIELD BU. PER ACRE	HEIGHT INCHES	YIELD BU. PER ACRE
0	117	81 ± 7	117	81 ± 7	117	81 ± 7
I-5	87	64 ± 11	81	51 ± 7	77	41 ± 5
6-10	97 ± 1	45 ± 12	84 ± 1	36 ± 5	82 ± 2	34 ± 4
11-15	97 ± 3	38 ± 4	84 ± 2	34 ± 3	83 ± 2	26 ± 2
16-20	88 ± 4	22 ± 4	85 ± 3	24 ± 3	75 ± 4	14 ± 3
21-25	81 ± 2	20 ± 6	75 ± 3	21 ± 3	71 ± 3	13 ± 2
26-30	92 ± 3	24 ± 9	80 ± 2	18 ± 4	77 ± 3	9 ± 4

FIGURE 1.—A comparison of three maize lines, derived from the same variety, self-fertilized for 30 generations. Height of stalk is measured in inches and yield of grain in bushels per acre, both plotted on the same scale. The broken lines are the theoretical curves of inbreeding explained in the text.

The other three lines, I-6, I-7, and I-9 have remained normal in every respect except for a much reduced rate of growth. Their behavior as measured by height of stalk and yield of grain during the 30 generations of self-fertilization is given in table 1 and figure 1. The results are averaged

for five-year periods to reduce the seasonal fluctuations. Wide variations, especially in yield, occur from season to season due to varying weather conditions. Since it is impossible to save seed of corn for more than a few years and have it produce normally there is no way to overcome these seasonal variations. A long continued experiment on inbreeding with plants is as much a record of weather conditions as it is of the effects of consanguinity. For example, the 1-6 line in generation thirty was grown both in 1936 and 1938 from the same lot of seed. In the former year it yielded at the rate of 38.3 bushels per acre and in the latter year 1.9 bushels. All yields were high in 1936 and extremely low in 1938 due to the rainy season and late maturity.

No measurements on height of stalk were made until the fifth generation. The original, naturally pollinated variety was grown in 1905 in Illinois and again in 1916 in Connecticut from seed obtained anew from the original source. The yields were 75 bushels of dry shelled grain in Illinois and 88 bushels in Connecticut. These two results are averaged to give the yield at the start before inbreeding. Height of stalk was measured only on the original variety grown in Connecticut in 1916. All yields have been calculated from air dried ears, 70 pounds of ear corn equalling one bushel of shelled grain.

By averaging the results for five-year periods some of the seasonal fluctuations are smoothed out and the general trend stands out clearly. The reduction in height of stalk has taken place almost entirely in the first five-year period. After the fifth generation no significant change has taken place, giving due consideration to the probable error as measured by the standard deviation calculated by Bessel's formula. Yield continued to decline in all three lines until generation twenty. During the last 10 generations no significant change has taken place. The broken lines in figure 1 are the theoretical reductions calculated in the following way: The average height and average yield of the three lines at the end of the 30 generations is subtracted from the figures at the start. This difference is halved in each generation and added to the end result.

In calculating the theoretical curves the results are averaged for five generation periods to correspond with the actual results treated in the same way. Since no height measurements were made until the fifth generation the theoretical height is not averaged for the first five generations. Only the one figure for the fifth generation is used. (Example: $117 - 83 = 34$. This number halved five times is 1.0625. Added to 83 it gives the theoretical height for the fifth generation. The theoretical yields for the first five generations, obtained in the same way, are 49, 35, 25, 21 and 19. The average of these is 29.4, the first point in the theoretical curve for yield.)

These expectations are based upon the assumption that the inbreeding

effect is correlated with the degree of heterozygosity as explained previously (JONES 1918a). The number of heterozygous allelic pairs is reduced 50 percent, on the average, in each generation of self-fertilization. The more rapid attainment of constancy in height than in yield may be understood on the assumption that height of stalk is less complex in its transmission than yield. Grain production sums up the plant's entire ability to grow and to reproduce and no doubt is influenced in its expression by many more inherited determiners than length and number of internodes.

In a previous publication (JONES 1924) results were given from two of these Leaming inbreds which were crossed, and successive selfed generations from the F_1 to F_8 grown, all in the same years. New seed was produced from a number of plants self-fertilized. Since the original plants were first generation hybrids of inbred strains the results are not directly comparable to the figures given above. For testing the effect of inbreeding upon yield they are better because seasonal fluctuations are eliminated to a considerable extent and the mixing of seed from several plants reduces the chances of using progenitors more heterozygous than the average. The yields in bushels per acre obtained compared to the theoretical expectations are as follows:

	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Actual	101	69	43	44	23	27	25	27
Expected	—	64	46	36	32	29	28	—

This is a remarkably close parallel considering the many external factors that can affect yield. The height measurements obtained from the same plants are not so closely in agreement with the theoretical expectation. They are as follows:

	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Actual	95	82	78	77	67	63	60	59
Expected	—	77	68	64	61	60	60	—

The slower reduction in any measurable character, than the theoretical curves indicate, could result from the survival and selection of the more vigorous individuals as progenitors. In this way the attainment of homozygosity is delayed. It is expected that inbred lines will behave differently. By random assortment some individual progenitors will be more heterozygous and some less than the average. Experience with a large number of inbreds indicates that the majority of them are reduced to a high degree of stability in less than ten generations.

Since the standard errors are so high there is no certainty that the reduction in yield and height after 30 generations has come to an end. Moreover this reduction may go on indefinitely even if the plants are now re-

duced to complete homozygosity, due to degenerative changes. There is good evidence to indicate that loss variations have occurred in these lines. In figure 2 the behavior of two lines is given when separated at various

FIGURE 2.—Two families separated into sib lines at various generations and continued until shown to be genetically similar or diverse. Both are plotted at the same scale in different positions.

stages of inbreeding. Height of stalk is used because it is less influenced by seasonal fluctuations than yield. The 1-6 line in the eighth generation was separated into two sib lines and grown in adjoining rows until the sixteenth generation. The two lines were clearly distinct as reported in a previous publication (JONES 1924). At the same time the 1-7 line was also separated and the two sib lines continued to the 17th generation.

FIGURE 3.—The 1-7Q1 line in the 29th generation of continuous self-fertilization.

As no significant difference appeared in these paired lines one of them was discontinued and another separation made in the other in generation 17. These two lines 1-7Q1 and 1-7Q2 have been continued to date and have been significantly different for this entire period in appearance as well as in measurable characters. The difference in ear type is shown in figures 3 and 4. The 1-7Q2 line has noticeably smaller and less well filled

FIGURE 4.—The 1-7Q2 line separated from the 1-7Q1 line in the 17th generation and further inbred for 12 generations.

ears. The production of grain is significantly less as shown by the results given in table 2. This poorer yielding line is taller in stalk growth. The

TABLE 2
Behavior of inbred lines of maize separated in the 17th generation and continued to the 30th generation of self-fertilization.

GENERATIONS SELFED	HEIGHT OF STALK—INCHES			YIELD—BUSHELS PER ACRE		
	1-7Q1	1-7Q2	DIFF. 1-2	1-7Q1	1-7Q2	DIFF. 1-2
18	77.3	80.5	-3.2	—	—	—
19	82.6	92.5	-9.9	—	—	—
20	84.4	93.4	-9.0	19.1	17.8	+1.3
22	70.1	78.0	-7.9	14.1	9.6	+4.5
23	73.0	76.5	-3.5	19.8	21.4	-1.6
24	74.8	81.3	-6.5	23.5	15.9	+7.6
25	81.8	89.7	-7.9	27.3	22.2	+5.1
26	79.1	85.9	-6.8	22.9	14.0	+8.9
27	76.6	77.9	-1.3	19.5	16.1	+3.4
28	76.7	81.3	-4.6	26.7	16.9	+9.8
29	88.6	94.2	-5.6	16.9	10.1	+6.8
30 1936	80.9	82.6	-1.7	31.3	25.3	+6.0
30 1938	75.6	73.4	+2.2	4.2	2.5	+1.7
Average	78.6	83.6	-5.0	20.5	15.6	+4.9

differences in height are all minus and in yield all plus, with one exception in each case, and the odds measured by Student's method are considerably less than one in 100 that these differences are due to chance alone.

FIGURE 5.—The 1-6V₁ line in the 29th generation of continuous self-fertilization.

FIGURE 6.—The 1-6V₂ line separated from the 1-6V₁ line in the 22nd generation and further inbred for seven generations.

The 1-6 line was also again separated in the 17th generation but as no differences were apparent in generation 20 one of these was discontinued. It was again separated in generation 22 and the two sib lines continued to date. Height measurements made in the 28th generation are given in table 3 and show a slight difference which is not statistically significant. Representative ears of these two sib lines are shown in figures 5 and 6.

A sensitive method for testing heterozygosity is to cross separated lines and compare the F_1 progeny with the two parental lines. This was done for all three lines separated after seven generations and continued for six or more additional generations (JONES 1924). The crosses of the paired lines were significantly increased in yield over the parents in the 1-6 and 1-7 lines but not in the 1-9 line. Sib lines of 1-6 and 1-7 separated in the 22nd and 17th generations and further self-fertilized for six and eleven generations were crossed with each other and the results for height and yield are given in table 3. The F_1 progenies do not differ significantly from

TABLE 3
The result of crossing inbred lines of maize that had been separated in generations 17 and 22 and further inbred for 11 and 6 generations.

PEDIGREE	HEIGHT OF STALK IN INCHES										YIELD			
	61	64	67	70	73	76	79	82	85	88	N	MEAN	S.D.	BU. PER ACRE S.D.
1-7Q1 K	8	5	10	2							25	65	3.0	27 3.8
F_1	1	13	19	12	3						48	67	2.8	25 1.2
1-7Q2 K		3	9	4	6	1					23	69	3.1	17 2.5
1-6V1 F					2	7	8	7	3	1	28	80	3.7	36 2.5
F_1				1	3	11	16	7	3		41	79	3.3	33 1.4
1-6V2 F			1	5	4	9	8	1			28	78	3.7	35 1.9

either parent. There is every indication that these lines have now been reduced to complete, or very nearly complete, homozygosity, at least for all loci that have any effect upon hybrid vigor.

The differences that have appeared in the last 15 generations are more probably due to germinal variations rather than to delayed segregation from an originally heterozygous complex. Two transmissible variations had previously appeared in these lines. Defective seeds were noted in the 1-6 line in the 13th generation and red cobs in the 1-7 line in about the tenth generation. This latter is a change from a recessive to a dominant condition.

Since these inbred strains are so uniform in all visible characters and so much reduced in size and vigor, any outcrossing which may take place is immediately noted in the much larger and more vigorous plants which result. None of the variations noted can be attributed to contamination.

The 1-6 line was the most vigorous and high yielding of all the lines grown in the first half of the inbreeding period and at the present time is the most productive. Representative ears of this line are shown in figures 5 and 6. Comparing these specimens with the ones shown in figure 7 for the seventh generation and in Connecticut Agricultural Experiment Station Bulletin 207, Plate IIA for the tenth generation it will be seen that

the ears are now somewhat shorter than they were in the early stages of inbreeding. Ears of this line in about generation 13 are shown in GENETICS 9: 405-418, 1924, figure 1. These are quite similar to the ones shown here produced in generation 29. (Previous photographs show selected ears. The illustrations given here include the entire crop from small progenies of about 20 plants each. Some of the small specimens are second ears.) In stalk growth and kernel type there has been no marked change since the tenth generation. This line now produces much aborted pollen apparently due to a failure of normal synapsis.

FIGURE 7.—The four lines after seven generations of self-fertilization.
(From photograph by H. K. HAYES taken in 1914)

The 1-7 line shown in figure 3 is considerably smaller in ear size than it was in generation 10. There is a noticeable tendency for the ears to be poorly filled at the tips with the production of many rudimentary staminate florets. While the cobs are still flattened they are not as broad as the ears shown in Bulletin 207, Plate IIb. The change in height of plant and ear type, shown by the sib lines separated in generation 17, is a germinal change of some kind. The reduction in size of ear and seed formation would be interpreted as a degenerative change if it were not that the plants are taller than the normal line which has not changed in ear type. Whether these changes involve more than a single locus has not been determined.

The 1-9 line has been the least productive of the three. It is a heavy pollen producer but is so late in maturing that its yield is often cut short by the end of the growing season. In 1938 it yielded practically nothing. No

ears are available for photographing and only a few immature self-pollinated seeds are on hand to continue this line. In 1937 it yielded at the rate of 21 bushels per acre which is considerably above its average for the past 15 years. In a good year the ears do not differ noticeably from the specimens shown in Bulletin 207, Plate IIb. (See also Inbreeding and Outbreeding, p. 130.) Ears of this line in about the fifth generation are shown in United States Department of Agriculture Bulletin 243, Plates III and IV, and in the seventh generation in figure 7. From the fifth to tenth generation there had been a remarkable change.

TABLE 4
*The behavior of three inbred lines of maize with respect to infection
by the smut fungus (Ustilago zeae) from generation 10 to 30.*

YEAR GROWN	PERCENT OF PLANTS INFECTED		
	1-6	1-7	1-9
1917	0	9.8	0.3
1920	10.0	13.8	5.7
1922	2.2	27.3	3.0
1923	7.4	69.0	0
1925	2.9	19.6	0.7
Average		4.5	27.9
1926	5.0	46.5	0
1928	5.0	63.0	0
1929	0	15.7	0
1930	0	52.4	5.6
1931	21.7	56.8	29.8
Average		6.3	46.9
1932	7.1	71.4	11.4
1933	3.9	43.6	18.3
1935	29.2	83.9	11.1
1936	0	11.3	—
1937	—	—	45.0
1938	3.8	24.2	0
Average		8.8	46.9
Total Average	6.5	40.6	8.7

In the tenth generation it was noted that these inbred lines differed in susceptibility to the smut fungus (*Ustilago zeae*). A preliminary report was made in 1918b. In table 4 are given the percent of plants infected from generations 10 to 30. The figures are arranged according to the years grown and not by generations since the fluctuation was mainly seasonal. Comparing the averages for five year periods there has been an increase in number of plants affected. The relative position of the three lines has remained about the same. The 1-7 line from the start has been the most severely injured. Not only do more plants of this line show the black fruit-

ing bodies of the fungus but these spore-bearing structures are larger and the plants show more injury. There are many barren stalks and some plants are killed before maturity. In this line the black spore masses usually appear at the nodes near the base of the plant or at the ear nodes. In the other two lines the fruiting bodies appear in the tassel or at the upper nodes. In these lines also the smut balls are usually small and the plants are seldom injured appreciably.

The increase in percent of plants infected seems to be one of the manifestations of the effect of inbreeding. As the plants are reduced in vigor they are less resistant. Since the increase in susceptibility has come after generation 20 at which time the reduction in yield is assumed to have come to an end this may not be a valid conclusion. The wide fluctuation in number of plants affected is largely seasonal. The years from 1931-1937 were a period of relatively high smut infestation.

No variations during the long continued inbreeding program have appeared that can be considered as favorable to survival. Usually not more than 100 plants of any one progeny have been grown each year, not a large number in which to look for variations. But many progenies have been grown and in the 10 years after uniformity and constancy had been reached there has been a reasonable chance for growth variations to appear. Because the plants are so uniform, slight variations would be noticeable and anything favoring growth would likely be selected. Since these inbred strains are so late in maturing any variation that helped the plants to ripen earlier would tend to be perpetuated. Possibly unconscious selection of favorable growth variations has counteracted to some extent the reduction due to inbreeding. But if there is any tendency of this kind it did not save two lines from extinction and there is no certain indication that any of the lines are now improving in any character.

SUMMARY

From four original lines of maize three have been continuously self-fertilized for 30 generations.

One original line and one sub-line, separated in the second generation, failed to survive. At least in one case this failure is due to a degenerative change which apparently could not be prevented by selection of normal plants as progenitors.

Reduction in height has ceased after five generations and in yield after 20 generations.

Sib lines separated at various stages clearly differed in some cases and remained the same in others.

These differences are considered to be the result of spontaneous transmissible variations and not the result of delayed segregation.

After 20 generations of self-fertilization these inbred lines appear to be uniform and constant for all visible characters and homozygous for all loci that have any effect upon hybrid vigor.

Throughout the entire period of inbreeding no variations have appeared which could be interpreted as being favorable to survival.

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