THE ATTAINMENT OF HOMOZYGOSITY IN INBRED STRAINS OF MAIZE

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INTRODUCTION

Six generations of continuous self-fertilization in the naturally cross-fertilized maize plant has been found to be usually sufficient to bring about a rather high degree of homozygosity. Plants which are vigorous, but highly variable at the start, become uniform and constant and markedly reduced in size and in the rapidity of growth when self-fertilized at random. When single plants are used as the progenitors of each successive inbred generation everything depends upon the constitution of these progenitors as to the results obtained. If there is extensive selection for vigorous individuals reduction in heterozygosity may be delayed. Differences in the rate at which homozygosity is approached are expected in different lines, but on the average, without selection, the decrease in heterozygosity theoretically follows the rule of halving the remaining differences in each generation until complete homozygosity is attained. Actual results agree fairly well with this expectation as has been shown previously and by results to be reported here.

After seven generations of self-fertilization a number of strains were tested for homozygosity, as reported in 1918, by comparing progenies from self-fertilized plants with those from intercrossed plants within the same strain (JONES 1918). On the average the crossed plants gave a slight increase in all characters measured. In some strains the increases were statistically significant, whereas, in other strains equally large decreases resulted from crossing, indicating that the test was greatly affected

by environmental influences. On the whole, any increase in size and vigor was slight when compared with the result of crossing unrelated strains.

Some of these same strains have now been brought to the seventeenth generation of self-fertilization. Originally all of the common types of maize were inbred, but only four of the original strains have been continued after the tenth generation and these have descended from a single variety, a yellow dent type known as Leaming.

In the eighth or ninth generations of self-fertilization each of these four strains was separated into two lines which have been continued to the present time. The behavior of these paired lines, continued seven or more generations after separation, gives an indication as to what extent homozygosity had been attained at the time of separation.

For convenience these four strains are now lettered A, B, C and D, the two paired lines within each strain being designated A₁, A₂, B₁, B₂, etc. The generations have not been kept alike in all of the strains on account of the failure to secure self-pollinated seed in some years. The pedigrees of these strains are given in table 1. The first number in each pedigree

TABLE 1

Pedigree relationship of four inbred strains of maize, each separated in the eighth or ninth generation and further self-fertilized from seven to nine generations.

STRAIN DESIGNATION	PEDIGREE NUMBER OF THE PROGENITOR IN EACH GENERATION																	GENERATION WHEN		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Separated	Crossed
A ₁	1	9	1	2	4	6	7	5	3	2	1	1	1	1	1	1			8	14
A ₂	"	"	"	"	"	"	"	"	"	6	2	1	1	1	1	1	1	1	8	15
B ₁	1	7	1	1	1	4	7	5	4	7	1	1	1	1	1	1	1	1	9	16
B ₂	"	"	"	"	"	"	"	"	"	"	5	2	1	1	4	1	1	1	9	16
C ₁	"	"	"	2	2	9	2	1	1	4	3	1	1	1	1	1	1	1	9	16
C ₂	"	"	"	"	"	"	"	"	"	"	1	1	1	1	1	1	1	1	9	16
D ₁	1	6	1	3	4	4	4	2	4	4	2	1	1	1	1	1	1	1	8	16
D ₂	"	"	"	"	"	"	"	"	"	"	5	5	2	1	1	1	1	1	8	15

designates the variety, seed of which grew the first plants to be self-fertilized. Following that, each single plant progenitor is given a number in each generation. It will be noted that the four strains came from three original plants numbered 6, 7 and 9. One strain was separated in the third generation. The two resulting strains are nearly as different as the other strains from separate plants at the start so that they can be con-

sidered as distinct strains. This shows that after two generations of self-fertilization a high degree of heterozygosity still remained, as would be expected.

HOMOZYGOSITY AFTER EIGHT GENERATIONS

Three of the paired strains, A, B and C, have remained visibly alike in the seven to nine generations they have been grown since separation. No consistent differences in general size, habit of growth, time of flowering, details of structure or disease infection can be seen in the field. However, the seeds of C_2 are appreciably brighter in color than those of C_1 although this difference is not clearly apparent every year. In the case of D_1 and D_2 , differences were apparent soon after they were separated and they have remained diverse up to the present time. The stalks of D_2 are larger, the leaves are wider and lighter in color. The ears are larger and the seeds are wider and duller in color. These differences are easily apparent during the growing season and at harvest time.

In table 2 the paired strains are compared statistically in height of plant, number of nodes, length of ear and in yield. These figures are

TABLE 2

A comparison of inbred strains of maize from the same sources, but separated after seven generations or more of self-fertilization.

	HEIGHT INCHES	NODES NUMBER	LENGTH OF EAR CENTIMETERS	YIELD IN BUSHELS PER ACRE
A_1	$60.8 \pm .47$	$13.98 \pm .074$	$10.28 \pm .15$	9.4
A_2	$61.5 \pm .39$	$14.25 \pm .079$	$12.06 \pm .11$	17.6
Difference	$.7 \pm .61$	$.27 \pm .108$	$1.78 \pm .19$	8.2
Diff. + P.E.	1.1	2.5	9.4	
B_1	$77.3 \pm .45$	$13.37 \pm .066$	$8.64 \pm .13$	28.2
B_2	$78.3 \pm .66$	$14.12 \pm .056$	$8.75 \pm .12$	27.3
Difference	$1.0 \pm .80$	$.75 \pm .086$	$.11 \pm .18$	.9
Diff. + P.E.	1.3	8.7	.6	
C_1	$74.7 \pm .60$	$15.43 \pm .068$	$11.16 \pm .24$	17.0
C_2	$73.1 \pm .52$	$16.02 \pm .063$	$10.09 \pm .24$	16.7
Difference	$1.6 \pm .79$	$.59 \pm .093$	$1.07 \pm .34$	.3
Diff. + P.E.	2.0	6.3	3.2	
D_1	$75.0 \pm .60$	$14.91 \pm .090$	$9.96 \pm .16$	19.8
D_2	$70.8 \pm .63$	$13.63 \pm .098$	$13.71 \pm .25$	23.7
Difference	$4.2 \pm .87$	$1.28 \pm .133$	$3.75 \pm .30$	3.9
Diff. + P.E.	4.8	9.6	12.5	

based on duplicate plots of each pair grown close together and representing a total of about 50 plants in each line. All of the paired lines are significantly different in some character but the D lines differ significantly in all characters measured.

The paired lines, after being separated for six or more generations, were crossed both ways and compared with their parental strains. If differences existed when the paired lines were separated, these differences should persist and might result in an increase in growth whenever they were crossed. In table 3 the average of the reciprocal crosses, each grown in duplicate plots, is compared with the average of the two parents, in height, number of nodes, length of ear and yield of grain.

TABLE 3

A comparison of inbred strains of maize from the same source, with their reciprocal intercrosses.

		HEIGHT INCHES	NODES NUMBER	LENGTH OF EAR CENTIMETERS	YIELD IN BUSHELS PER ACRE
A	Parents	61.2 ± .30	14.1 ± .05	11.2 ± .11	13.5
	Hybrids	63.7 ± .29	14.5 ± .05	11.6 ± .08	15.8
	Difference	2.5 ± .42	.4 ± .07	.4 ± .14	2.3
	Diff. ÷ P.E.	6.0	5.7	2.9	Odds = 7:1
B	Parents	77.8 ± .40	13.7 ± .06	8.7 ± .09	27.8
	Hybrids	80.6 ± .30	13.8 ± .04	9.2 ± .09	33.4
	Difference	2.8 ± .51	.1 ± .08	.5 ± .13	5.6
	Diff. ÷ P.E.	5.5	1.2	3.9	Odds = 158:1
C	Parents	73.8 ± .40	15.8 ± .05	10.6 ± .18	16.8
	Hybrids	72.2 ± .48	15.8 ± .05	11.9 ± .14	25.4
	Difference	-1.6 ± .62	0 ± .07	1.3 ± .23	8.6
	Diff. ÷ P.E.	2.6	0	5.7	Odds = 144:1
D	Parents	73.0 ± .46	14.3 ± .08	11.7 ± .20	21.8
	Hybrids	80.8 ± .40	14.7 ± .06	13.4 ± .15	38.2
	Difference	7.8 ± .61	.4 ± .10	1.7 ± .25	16.4
	Diff. ÷ P.E.	12.8	4.0	6.8	Odds = 78:1

The probable error for height, number of nodes and length are calculated from the usual formula for the spread of a frequency distribution. Since yield is based only on the average results of paired plots grown in different parts of the field the significance of the differences is calculated from the method (STUDENT 1908, LOVE 1923) which gives the odds

against such a difference occurring in repeated experiments. These odds are given in table 3 and the increases in yield of the reciprocal crosses over their seed parents are surely significant in all cases except for the A lines.

Wherever there is a significant difference it is an increase of the hybrids over their parents. The crosses in strains A, B and C, do not show a significant increase in all characters measured, so that the differences between the paired lines in each strain are presumably small. In these strains, it will be recalled, no visible differences in size or form could be seen. In the D strain, where noticeable differences existed in the paired lines, the hybrids exceed their parents in all characters measured, by a significant amount. The production of grain is nearly doubled. Height of plant and length of ear are augmented by over 10 percent.

Since the two D lines were different from the time they were separated in the eighth generation, there is no question but that their common ancestor in the seventh generation was still heterozygous. Whether or not all the differences can be attributed to delayed segregation can not be positively stated. There is the possibility based upon direct evidence that germinal changes have occurred in all of the strains, making them different to some degree even when no external differences are apparent.

HOMOZYGOSITY AFTER FOURTEEN GENERATIONS

With the evidence that the strains were not reduced to complete homozygosity by eight generations of self-fertilization the question arises as to how nearly they have approached this condition in later generations. This was tested in the following way. Two different strains, A and B, in which the paired lines did not differ visibly, were crossed in the fourteenth generation for the A strain and the fifteenth generation for the B strain. The F_1 plants of $A \times B$ were remarkably uniform and vigorous. Several F_1 plants were self-fertilized and an equal number of plants was pollinated by other plants of the same lot. If the parental strains were not homozygous the F_1 plants would therefore not be all alike and intercrossing these somewhat diverse individuals would not cause as great a reduction in growth the following generation as self-pollinating them. On the other hand if the parental strains were completely homozygous the F_1 plants would all be exactly alike in germinal construction and cross-fertilization among the hybrid offspring would give no different result than self-pollination. This test for homozygosity was first applied by SHULL (1909, 1911) to inbred strains of maize self-fertilized four generations. He found that the F_1 plants intercrossed gave an increase of 13 percent

in production of grain, indicating that the parental strains were not completely homozygous.

The seed ears from the (A×B) F₁ plants, self-fertilized and intercrossed, were equally well developed. The seed of several ears of each lot was mixed and the two lots were planted in alternate rows, replicated three times with guard rows on the outside. Enough seed was planted and the extra plants thinned out to insure a practically perfect stand of plants. The F₁ plants self-pollinated gave a progeny which averaged $76.2 \pm .57$ inches in height compared with $73.8 \pm .70$ for the F₁ plants intercrossed, a difference of $2.4 \pm .90$ in favor of the self-pollinated plants. In yield the results were, respectively, 22.2 ± 1.2 and 22.0 ± 2.4 bushels per acre, a difference less than the probable error of the difference.¹ Since there was no significant difference in height or production of grain, the two characters which are most influenced by heterosis, the two parental strains are apparently each homozygous, as far as this test shows, for all factors which have a measurable effect upon growth.

MUTATIONS IN INBRED STRAINS OF MAIZE

Two new characters have appeared in these four longest-inbred strains, in such a way as to leave little doubt that they are germinal changes and not due to delayed segregation. Since inbred strains of maize, after the first several generations, are uniform in type and much reduced in vigor, changes occurring in any part of the plants are easily noted. Moreover, they can be clearly distinguished from alterations due to out-crossing. Crossing with any plants outside the strain results immediately in an enormous increase in size and rapidity of growth and change in form so that any out-cross is at once noted. Both of the mutational changes in these inbred strains occurred without the plants increasing in vigor or changing in type other than in the character affected.

In 1921 the D₂ strain was in the thirteenth generation of self-fertilization. Three self-pollinated ears were obtained and one of these was clearly segregating for a lethal factor which produced poorly developed, shrunken, light-colored, opaque seeds scattered throughout the ear as shown in figure 1. Heritable characters of this kind, described as defective seeds, have been found to occur quite commonly in all kinds of maize. They were first noticed in 1918 in other inbred strains three years before they were found in the old inbred strains. Since variations of this kind

¹ The triplicate plots were grown in one place and the probable error of yield is here calculated from BESSEL's formula, $E_A = .6745 \sqrt{\frac{\sum d^2}{n(n-1)}}$ (see LOVE 1923).

were being actively looked for it is very improbable that they could have been occurring in this strain each year and overlooked. It happens that the year before, all of the plants of this particular strain were hand-pollinated, some 15 to 20 plants in each of the paired lines, D_1 and D_2 , and if this character were present in the stock it would surely have been noted.

Defective seeds have reappeared in this mutant line every year since, now three generations in succession, and segregating plants out-crossed to

FIGURE 1.—A germinal change which occurred in the thirteenth generation of self-fertilization. The ear on the right is segregating for a lethal factor that produces defective seeds; on the left is a normal ear from the same progeny.

unrelated plants not segregating gave the same type of defectiveness in the second seed generation so that there is clear evidence that the lethal factor is recent in its expression and is heritable.

In 1922 about one-thousand plants of one of the C strains, after more than ten generations of self-fertilization, were grown in an isolated field and detasseled to produce seed crossed by another inbred strain. At

harvest four ears were found with light-red cobs. This strain has always had only pure white cobs and these are characteristically flattened. The ears and plants of this strain are easily identified and are quite distinct from all other inbred strains. The four plants which produced the colored cobs were noted at harvest as being typical for the strain and the ears themselves were no different from the others of the same lot except in the color of the cob. The change therefore can not have been due to out-crossing.

The seeds produced on these aberrant ears were all crossed with a red-cobbed strain before they were found, so that the next-generation plants would have colored cobs in all cases. As yet it has not been possible to test out the new character to determine its inheritance. If it is the same factor as the one that commonly produces red cobs the change has been from a recessive to a dominant condition.

Two other changes have occurred in other inbred strains of maize such that they have every evidence of being recent germinal alterations. One strain, after five generations of self-fertilization, produced, for the first time, striped and variegated plants which occurred regularly thereafter in about 25 percent of the offspring from normal plants. Another strain, after nine generations, gave small, narrow-leaved dwarf plants which segregated very clearly from the normal plants. This latter character has been out-crossed and again recovered, so it is a heritable change.

Abnormalities which are inherited as simple Mendelian recessives are continually being found in maize. Presumably they are due to mutation, but since they can be easily carried along in heterozygous stock there is no way of knowing when the new character actually originated. In these changes in inbred strains we have clear evidence of the occurrence of heritable variations in maize at a definite time.

Since changes in visible characters have occurred, it is possible that other variations have taken place which affect the external appearance of the plants so little that they can not be easily noted, but which at the same time make individuals from the same source germinally unlike and result in an increase in growth when the plants are crossed. On account of the likelihood of germinal changes taking place together with delayed segregation due to balanced lethals and other factorial and chromosomal complications, it is probably impossible to obtain strains which are absolutely homozygous. Before self-fertilization automatically reduces a heterozygous stock to complete uniformity and constancy, germinal changes will occur which will make some individuals heterozygous with respect to some factor pairs. From this it is evident that homozygosity

is entirely a matter of degree and that the complete state is rarely if ever attained.

INBREEDING AFTER CROSSING INBRED STRAINS

The behavior of long-inbred strains, when crossed and again inbred, demonstrates very clearly the working of hybrid vigor. After eight generations of self-fertilization two of the Leaming strains, A and B, were crossed. The first-generation hybrid was self-fertilized and each following successive inbred generation from this cross was again self-fertilized until seven inbred generations were secured. These successive inbred generations together with parental strains and their first-generation hybrid were all grown in adjoining rows each year from the time the two strains were first crossed. Single plants self-pollinated at random were used as the progenitors in each generation. The original seed was used up to the fifth generation after the cross. In that year single plants were again self-pollinated in each generation to give a fresh supply of seed. This was necessary because maize does not retain its viability and vigor long enough to complete the demonstration. The character of the progeny has depended upon the particular plant chosen as the progenitor and the results have been somewhat erratic since each generation does not have the same particular ancestor the previous generation has had. Somewhat smoother results might have been obtained by self-pollinating a large number of plants and using a composite sample of seed.

In 1923 seven successive inbred generations together with the first-generation hybrid and the two parental strains were grown in adjoining rows. Representative plants are shown in figure 2 in the order they grew in the field, making a natural curve of inbreeding. The data for average height obtained from about 50 plants in each lot are shown graphically in figure 3.

The data for length of ear for the same plants are given in figure 4. Measurements for height and length of ear are given for the one year, 1923. The production of grain was measured every year except one following the original cross, each year having one more generation than the previous. The yields obtained each year are given in table 4. The average production during six years for the parental strains, the F_1 and F_2 generations, with the later generations, each one year less, is shown graphically in figure 5. The production of grain is much more variable than the other characters measured, since yield is easily influenced by all environmental factors. The greater susceptibility of some generations to smut infection than others has been one reason for the somewhat erratic

results. The decrease in rate of growth after crossing is shown very clearly in figure 6.

The reduction in variability from the F_2 to the F_8 generation was very noticeable in the field. One of the parents has colored silks the other

FIGURE 2.—Representative plants from two long-inbred strains at the left, their first generation hybrid adjoining, followed by successive self-fertilized generations from this hybrid. Grown in adjoining rows in the same year.

FIGURE 3.—Height of plant in successively self-fertilized generations of maize.

TABLE 4

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

YEAR GROWN	GENERATIONS									
	P_1'	P_1''	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
1917	5.5	21.5	64.5	56.0						
1918	23.5	26.9	120.6	128.4	15.1					
1920	27.8	16.2	127.6	47.6	34.8	29.0	9.9			
1921	13.1	19.6	72.8	54.5	49.2	32.7	15.3	23.1		
1922	26.1	20.0	160.4	83.2	73.7	67.5	48.8	35.7	22.7	
1923	21.0	13.2	61.0	45.0	40.5	47.0	15.8	23.0	26.2	27.2
Average	19.5	19.6	101.2	69.1	42.7	44.1	22.5	27.3	24.5	27.2

Pedigree..... P_1' P_1'' F_1 F_2 F_3 F_4 F_5 F_6 F_7 F_8
 Generations selfed.....17 16 0 1 2 3 4 5 6 7
 Earlength in cm..... 8.4 10.7 16.2 14.1 14.7 12.1 9.4 9.9 11.0 10.7

FIGURE 4.—Length of ear, in centimeters, in successively self-fertilized generations of maize.

uncolored. The F_1 plants were all colored. The F_2 , F_3 and F_4 generations segregated for 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 F_1 generation. The plants in the F_2 to F_6 generations were quite variable but later became more and more uniform, until the last two generations were as uniform as the two parental strains although differing from both in type. The behavior with respect to variability in the several

FIGURE 5.—Yield of grain, in bushels per acre, in successively self-fertilized generations of maize.

FIGURE 6.—Daily increase in height in inches, in successively self-fertilized generations of maize.

generations, in height of plant, is shown graphically in figure 7.² The low variability of the F₄ generation is an exception to the otherwise regular reduction in variability.

² The two parental strains in 1923 were quite variable in height, due to some unfavorable environmental influence in the early seedling stage, which did not affect the other generations so noticeably. Since these long-inbred strains had always been exceedingly uniform previously, the data used in figure 7 for the two parental strains are from plants grown in 1922.

Pedigree.....	P ₁ '	P ₁ ''	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈
Generations selfed.....	17	16	0	1	2	3	4	5	6	7
C. V., height of plant.....	5.2	9.6	6.4	11.8	11.1	5.7	8.0	7.6	5.9	5.2

FIGURE 7.—Variability in height of plant in successively self-fertilized generations of maize.

DISCUSSION

This demonstration of the results of inbreeding, crossing and again inbreeding, conforms with the interpretation of hybrid vigor based upon the known facts of Mendelian heredity and chromosome behavior. Inbreeding a heterozygous stock automatically sorts out uniform and fixed types which, in the case of maize, are much reduced in size and vigor. Crossing any two of these fixed types, descended from different individuals at the start, results immediately in an increase in size and rapidity of growth. Uniformity is retained in the first generation but lost in the second. Further inbreeding again reduces size and vigor in the same way it did the first time; uniformity and fixity of type are regained at about the same rate on the average, until, in approximately the same number of generations of self-fertilization, the same level of vigor is reached. Due to recombination the resultant strains differ from their parental strains in varying degree. Since the rate at which constancy is approached may differ with the degree of heterozygosity of the progenitors chosen in each generation and their constitution with respect to specific growth factors, it is remarkable that the inbreeding process follows the same general course so closely as it does.

SUMMARY

Four strains of maize, self-fertilized eight generations or more, were each separated into two paired lines which were further self-fertilized for eight or nine generations more. The paired lines of two of these strains at the end of this period did not differ visibly in any respect. One strain differed

only in seed color while the fourth was noticeably different in many characters.

When the paired lines were crossed and the hybrids compared with their parents, there were significant increases in some characters in the strains which were visibly alike. The hybrids of the paired lines which differed in many respects were significantly increased in all characters.

After fourteen generations of self-fertilization in one strain and fifteen in another, a test for homozygosity was made by comparing the progeny from self-fertilized F_1 plants of the cross of these two diverse strains, with the progeny from intercrossed F_1 plants. No significant difference in height or production of grain was obtained.

Two germinal changes have occurred in these long-inbred strains, in such a way that they could not be due to out-crossing and were probably not due to delayed segregation.

Self-fertilizing plants produced by the crossing of two long-inbred strains, in seven successive generations, again brought about a reduction in size and rapidity of growth, and an increase in uniformity, which proceeded at approximately the same rate as when the plants were first inbred, until about the same level of vigor was established, at which time the reduction practically ceased.

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