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JOURNAL OF AGRICULTURAL RESEARCH

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HYBRID VIGOR AND WEIGHT OF GERMS IN THE SEEDS OF MAIZE [1]

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INTRODUCTION

The stimulation of growth resulting from hybridization remains one of the unexplained biological facts. The fact itself has been recognized from very early times and is the basis of the current effective system for improvement in maize and other crop plants. But just what takes place in the union of unlike germ plasms that brings about the observed stimulation has not been determined. The exact nature of the reaction between diverse hereditary elements is of great theoretical as well as practical interest, and until this problem has been solved all facets of hybrid vigor will continue to merit attention.

PREVIOUS INVESTIGATIONS

The demonstration of hybrid vigor in resting embryos, as distinct from seed size, was made by Ashby (*1*, *2*).[2] Using maize as his test plant, he investigated the possibility that the increased size of the mature hybrid organism over that of its parents was determined by the weight of the embryo and not by an increased rate of growth of the developing hybrid plant over that of the parents. Ashby concluded that hybrid corn plants grew at the same rate as their more rapidly growing parent and that their larger mature size followed from the fact that they had a larger embryo to start with, i. e., more capital invested. He subsequently extended his studies to other species and reached the same conclusions (*3*).

Ashby's conclusions as to the nature of hybrid vigor have been criticized by East (*7*) and amplified by Ashby (*4*, *5*). There can be no difference of opinion as to the facts. Hybrid embryos are often heavier than those of their parents. The divergence of opinion comes with the question as to whether all the increase in mature size of a hybrid over its parents can be attributed to the initial advantage of a larger embryo and, therefore, to a higher rate of growth, limited to the period before dormancy.

Obviously, if hybrid embryos produced on inbred plants are heavier than inbred embryos on the same or sister plants, they must grow either for a longer period or at a more rapid rate if growth is under-

[1] Received for publication May 27, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 80.

stood to mean an increase in dry weight, whether resulting from larger cells or more of them.

Lindstrom (*8*), using mutilated maize plants and failing to grasp Ashby's main thesis, believed he had demonstrated that the larger size of hybrid plants was not the result of a greater initial capital.

Sprague (*12*) showed, with some of the same seed stocks as Ashby, that reciprocal crosses differing in germ size attained identical final weights. He also pointed out the fallacy of Lindstrom's reasoning and reported a cross where the embryos were no heavier than those of one of the parent inbreds, yet the final weight of the hybrid plant was twice that of the inbred plant. Sprague concluded that the rate of growth of hybrids in the earlier stages of development was more rapid than that of the inbreds and that the increased final size was compounded of a more rapid growth rate and a larger initial embryo size. He believed his experiments were more readily explained on the basis of dominant favorable genes than on the more subtle physiologic stimulation resulting from the union of unlike germ plasms.

Paddick and Sprague (*10*), experimenting with maize, measured the relation of increase in hybrid germ weight, over that of the female parent, to the resulting forage and grain weights. Twenty-four hybrids were tested in 1 year and 22 in another. There was a low correlation of germ-weight increase with grain yield both years ($r=0.42$ and $r=0.36$) but no correlation with yield of stover.

These authors also measured the variation of kernel parts of an inbred line of maize and of several hybrids with it. They pointed out that, irrespective of seed size (which varies with position on the ear and with years), the ratio of germ to endosperm remains relatively constant.

Evidence was also presented of very great differences between reciprocal crosses in weight of seed parts. Of the nine reciprocal crosses tested, there were five hybrids that had germs lighter in weight than those of the female parent, and four of these were below both parents in germ weight. These hybrids, therefore, although presumably giving the customary mature-plant increases in weight, showed no evidence of heterosis in their germs.

Passmore (*11*), working with *Cucurbita pepo*, where seed size and embryo size correspond and there are no complications with endosperm, showed that where reciprocal F_1 hybrids differed by two to three times in germ weight the resulting plants were not greatly different at maturity. However, the heavier seeds gave a decided initial advantage, which became less with time. The final differences were equalized partially, because the plants from the small embryos matured later. Passmore made no direct statement as to the effect of heterosis on embryo size but left the inference that there was none. She stated, "Since the size of the F_1 seed is determined by the maternal parent, it is possible, in a reciprocal cross, to get two hybrids which are genetically identical but which have widely different seed sizes." Probably, in view of the context, the determination of seed size is not wholly by the maternal parent, as it would seem strange were the effect of hybrid vigor not exhibited in the embryo of squashes.

Murdoch (*9*), improving on Ashby's technique of dissection of the maize embryo, confirmed the results of Ashby as to weight of embryos but apparently overlooked Sprague's results, which first proved that hybrids did not always have heavier embryos than their parents. He believed that one reason for the lack of accord between Ashby and his critics resulted from a failure to understand what is meant by the term "embryo." For his study, which was limited to embryo size, Murdoch removed the scutellum, the coleorhiza, and the endosperm.

It is true that the scutellum, coleorhiza, and endosperm do not become part of the mature corn plant, and for the purpose of calculating the rate of growth of the plant following germination these parts should not be considered as capital tissue. However, from the standpoint of the effect of hybrid vigor, each of these parts should respond alike to heterosis, as they are the products of fertilization. There is a cytological reason for discounting the response of the endosperm to cross-pollination, because the female parent plays a disproportionate part in the development of this tissue, furnishing two sets of genes to one from the male. In the case of the embryo tissue, comprising the scutellum, coleorhiza, and the embryo proper, both parents contribute equally, and indeed the scutellum and the coleorhiza are as much parts of the embryo as are the leaf and root primordia. They do not increase in cell number as the seedling expands and in a strict sense are not part of the capital. Their size, however, at the resting stage must be considered as much the result of heterosis as that of the primordia which are to become the next stages of the plant. It should be kept in mind that the cells of the mesocotyl (epicotyl), coleoptile, and indeed those of part of the leaves and roots, are formed before the resting period, and that the elongation of these organs, together with some part of their increase in dry matter in the early stages of growth, is in the nature of cell expansion. Roughly it might be estimated that half the increase in length of mesocotyls and coleoptiles results from the expansion of cells formed before the resting stage of the embryo.

Although the scutellum may not increase in dry weight with the growing plant, it is an indispensable part of the seedling, without which the embryo could not resume growth. This organ, therefore, should not be disregarded in considering the effect of hybrid vigor on embryo weight. Furthermore, it is quite likely that the weight of the scutellum increases with the resumption of growth after the resting period and that this weight is properly a part of the total dry weight of the seedling, even though it has not resulted from further cell division. The starch of the endosperm is not essential, as excised embryos will grow without it.

Ashby (*2, p. 1010*) gave data for the weights in milligrams of seeds, embryos, scutella, and total embryos for two parents and the F_1 and F_2 of maize, the seed of which was furnished by F. D. Richey. From the table, by subtraction, the endosperm weight can be derived. Taking the mean of the two parents as a base, it is found that the F_1 hybrid exceeds the parents in weight of grain by 14 percent, in weight of embryo by 58 percent, in weight of scutellum by 67 percent, and in weight of endosperm by 8 percent.

From the above data it is evident that the embryonic tissue represented by the scutellum increases in weight over the mean of the parents in the same order as does the embryo. The errors are such that the difference between these two parts of the embryo in percentage increase is not significant.

The relation of increase in weight of embryo to increase in weight of endosperm should throw some light on the nature of hybrid vigor. If dominant favorable growth factors are the sole explanation and dominance is complete, then the embryo and endosperm should show similar increases in weight, as the additional set of genes contributed by the female parent to the endosperm would have no more effect than the single set contributed to the embryo. On the other hand, if the favorable growth factors are not dominant, then the increase in weight of the embryo should be twice that found in the endosperm. This follows if it is assumed that each set of genes in both the endosperm and embryo produces an equal effect on weight, and further, that the genes derived from the two parents produce additive increases. Adding one set to the endosperm having unit effect would result in a 50-percent increase in weight, whereas a similar set added to the embryo would result in a 100-percent increase in weight.

Paddick and Sprague (*10, table 3*) give data that show an almost perfect agreement in the magnitude of percentage increase or decrease in weight of hybrid endosperms and germs. Only two real discrepancies were found, namely, hybrids A 12 × A 55 and A 25 × A 55. In the first of these hybrids the endosperm exceeded the female parent in weight by 34 percent, while the embryo showed only a 2.8 percent increase. In the second hybrid the endosperm exceeded the female parent in weight by 5 percent, while the embryo showed a 27.7 decrease. In their table 2, where 14 paired individual seed comparisons between hybrids and the female parent are shown, there are a number of wide differences between endosperm and embryo in their percentage increase in weight over the female parent. There is only one instance, however, where the hybrid germ shows a greater percentage increase in weight than the endosperm, and this is too small to be of significance.

Brink and Cooper (*6*) found in the case of alfalfa that the cells of the embryo increased in arithmetical progression for the first 6 days after fertilization. These authors also concluded that the rate of increase in number of cells in the embryo was no greater in crosses than in selfs. On the other hand, the nunber of nuclei in the endosperm during the same 6-day period increased exponentially, and the rate of increase in number was very much greater in crosses than in selfs. The authors point out that under these conditions hybrid embryos are accompanied by more advanced endosperm than inbred embryos. This circumstance might explain the greater size of hybrid embryos at the resting stage if it meant that greater endosperm activity resulted in better nourishment for the embryo. Seven plants were utilized in this study, and, although the crosses were between unrelated plants within the group, it is not clear whether the genetic backgrounds were such that heterosis was to be expected.

MATERIALS AND RESULTS

GERM WEIGHTS OF HYBRIDS AND PARENTS

Some of the seed stocks used by Ashby (*1*) in his original tests were furnished him by the writers and these have been used to check his results. The cross was between an inbred line of Pipe, self-pollinated for 12 years, and an inbred line of Pawnee, self-pollinated for 20 years. Crossed and self- or sib-pollinated seeds were produced together on the ears of Pipe and separated by means of differences in aleurone color. Two crosses were tested, both having the same Pawnee parent but differing in that in one case the female Pipe parent was self-pollinated whereas in the other the Pipe parent was pollinated with a sib plant. No seed was obtained from the selfed Pawnee male parent. The results are shown in table 1.

TABLE 1.—*Mean weight of whole seeds and endosperm and embryo fractions for single plant pollinations*

Progeny	Air-dry weight of whole seed	Oven-dry weight of—		
		Whole seed	Endosperm	Embryo
SELFED OR SIBBED FEMALE AND COMPARABLE HYBRID SEED ON SAME EARS				
	Gram	*Gram*	*Gram*	*Gram*
Pipe 194×Pawnee	0.2467±0.0021	0.2336±0.0022	0.2125±0.0019	0.0211±0.0004
Pipe 194 selfed	.2007± .0016	.1882± .0014	.1743± .0015	.0139± .0002
Pipe 195×Pawnee	.2112± .0012	.1986± .0013	.1812± .0013	.0174± .0009
Pipe 195×Pipe 194	.1709± .0089	.1598± .0094	.1481± .0081	.0117± .0007
SELFED AND HYBRID SEED ON SEPARATE EARS				
Pawnee selfed	0.2129±0.0061	0.1923±0.0055	0.1731±0.0051	0.0192±0.0006
Pipe×Pawnee	.2765± .0028	.2519± .0026	.2291± .0031	.0228± .0006
Pipe×Pawnee	.2902± .0056	.2635± .0049	.2387± .0045	.0248± .0008
Pipe selfed	.2955± .0031	.2662± .0026	.2459± .0029	.0203± .0004

Where hybrid seeds are borne on the same ears with selfed seed the increase in weight of endosperm is 22 percent and in that of embryo 52 percent (table 1). These increases also are found where hybrid seed is borne on the same ear with sib-pollinated seed, the endosperm of the hybrid exceeding that of the inbred by 22 percent and the hybrid embryo exceeding the inbred by 49 percent.

Two other hybrids between these inbred lines were examined. In these two cases selfed seed was not borne on the ears with the hybrid seed. The comparison involves three plants of the inbred Pipe, two having been crossed with Pawnee and one self-pollinated. The results are shown in table 1.

Both hybrids had endosperms lighter in weight than that of their female parent, and the embryos were only 12 percent and 22 percent heavier, respectively, than that of the female parent. Grown to maturity, these Pipe × Pawnee hybrids far exceeded their inbred parents in yield.

A set of sister plants was used to test the increase in weight of plants at various periods after planting. These crosses, because of the small number of seeds, could not be used to test both germ weight and growth increase. Seed weights were recorded and are shown in table 2.

TABLE 2.—*Mean weight of whole seeds and estimated weight of embryo for single plant pollinations*

[Selfed female and hybrid seed on same lines]

Progeny	Air-dry weight of whole seed	Estimated dry weight of embryo	Progeny	Air-dry weight of whole seed	Estimated dry weight of embryo
	Gram	*Gram*		*Gram*	*Gram*
Pipe 181 selfed	0.199±0.003	0.0136	Pawnee 521 selfed	0.198±0.014	0.0178
Pipe 181 × Pawnee 524	.225± .006	.0187	Pipe 182 selfed	.306± .012	.0210
Pawnee 524 selfed	.205± .018	.0185	Pipe 182 × Pawnee 523	.313± .022	.0260
Pipe 192 selfed	.220± .008	.0151	Pawnee 523 selfed	.244± .013	.0220
Pipe 192 × Pawnee 521	.258± .011	.0214			

From the data presented in table 1, it is seen that the embryos of self- or sib-pollinated Pipe plants are 6.9, 6.8, and 6.9 percent of the air-dry weight of the seed; those of Pipe × Pawnee are 8.6, 8.2, 8.2, and 8.5 percent; and the weight of the embryo of the single Pawnee given is 9 percent of the air-dry seed weight. From other examples not given here of seed weights of sister ears of these lines for this year (1935) it can be safely assumed that these percentages hold reasonably well. They have been used, therefore, to estimate the approximate germ weights of the seeds grown to obtain information on the rate of increase in plant weight. The estimated weights, based on percentages of 6.85, 8.3, and 9, are given in table 2.

These hybrids are identical with those reported by Ashby, though made 4 years later. The parents, therefore, had four generations more inbreeding. The estimated increased weights of hybrid germs over those of the female parent of 37.5, 41.8, and 24.4 percent compare with sister ears actually weighed and presented in table 1 of 52, 49, 12, and 22 percent and with Ashby's (*1*) material of 18.5 percent.

The field experiment was planted May 20, 1935, in 11 Latin squares, 3×3, planted in three rows, with five seeds 2 inches apart in each hill. The choice of seed and blocks was random. Eliminating the variance due to rows, columns, and means of Latin squares did not improve the accuracy of the test, and since the differences between means of sorts are large, the use of a generalized error derived from the analysis of variance is of doubtful propriety.

The errors, therefore, are estimated from the deviations of the plants of each sort from the mean of that sort for a given sampling date.

The mean weights in grams of the entire plant for the several sampling periods are given in table 3.

TABLE 3.—*Mean oven-dry weight of plants of the inbreds Pipe and Pawnee and the F_1 hybrid between them for various periods*

[Planted May 20, 1935]

Date of sample	Oven-dry weight of entire plant		
	Pipe	Pawnee	F_1 hybrid
	Grams	*Grams*	*Grams*
June 5	0.145±0.009	0.177±0 10	0.274±0.015
June 12	.395± .029	.428± .042	1.213± .056
June 19	1.687± .188	2.126± .287	7.407± .480
June 26	4.638± .044	7.655±1.451	21.273±1.549

The rates of increase for the three periods of 7 days each are given in table 4, and also the significance of the differences in rate of increase between the hybrid and each parent.

TABLE 4.—*Mean rate of increase in dry matter for the inbreds Pipe and Pawnee and the F_1 hybrid between them and differences in rate of increase between hybrid and each parent*

RATE OF INCREASE [1]

Period	Pipe	Pawnee	Hybrid
June 5 to 12	2.73±0.26	2.42±0.27	4.42±0.32
June 12 to 19	4.28± .57	4.96± .83	6.11± .49
June 19 to 26	2.75± .31	3.60± .84	2.87± .28

DIFFERENCES IN RATE OF INCREASE

Period	Hybrid−Pipe	Diff./S.E.	Hybrid −Pawnee	Diff./S.E.
June 5 to 12	1.69±0.41	4.1	2.00±0.42	4.7
June 12 to 19	1.83± .75	2.4	1.15± .96	1.2
June 19 to 26	.12± .42	.3	−.73± .89	.8

[1] Ratio of later to earlier value, from data given in table 3.

It will be seen that for the first period (June 5–12) the rate of increase of the hybrid was significantly larger than that of either parent. In the second period, while the rates of increase were all larger than in the first period, perhaps due to better growing conditions, the superiority of the hybrid declined, becoming of somewhat doubtful significance. And in the third period all differences were without significance, the inbred Pawnee actually making a slightly greater increase than the hybrid. The results of this experiment support those of Ashby and Sprague, but it should be noted that the errors of rates are such that differences of less than approximately 30 percent cannot be established. A 30-percent difference in rate would be greater than necessary to produce most of the observed increases in size of hybrids over their parents, irrespective of any differences in initial weight of meristematic tissue.

The data suggest that whatever factors led to the production of heavier embryos before dormancy operated also in the early seedling period.

A second series of F_1 crosses between inbred lines was examined. These lines are wholly unrelated to the Pipe and Pawnee lines and were inbred for only seven generations before the crosses were made. All pollinations were controlled, but each crossed and selfed line involved many plants. The seed was bulked, and 10 samples of 10 seeds each were drawn from the thoroughly mixed stock. The mean weights of various parts of the seeds are shown in table 5.

TABLE 5.—*Mean weight of whole seed and endosperm and embryo fractions for multiple plant pollinations*

[Selfed and hybrid seed on separate ears]

Inbred lines and hybrids	Air-dry weight of entire seed	Oven-dry weight			Weight of embryo [1]
		Entire seed	Endosperm	Embryo	
	Gram	*Gram*	*Gram*	*Gram*	*Percent*
Line A	0.3092±0.0084	0.2772±0.0084	0.2597±0.0084	0.0175±0.0006	6.3
A×B	.3652± .0065	.3394± .0058	.3177± .0057	.0217± .0012	6.4
Line B	.1853± .0037	.1679± .0034	.1555± .0031	.0124± .0005	7.4
C×A	.1984± .0017	.1843± .0017	.1738± .0017	.0105± .0004	5.7
Line C	.2180± .0035	.1987± .0031	.1822± .0009	.0165± .0007	8.3
Line D	.2530± .0026	.2291± .0023	.2140± .0024	.0151± .0006	6.6
B×D	.1765± .0058	.1615± .0054	.1486± .0052	.0129± .0039	8.0
D×B	.2903± .0060	.2659± .0058	.2482± .0054	.0177± .0006	6.7

[1] Measured as percentage of dry weight of seed.

As the cross showing the greatest increase in germ weight (A×B) proved to have the highest field yield and that showing no heterosis effect (C×A) proved to have the poorest yield, there is some evidence in this experiment to support the hypothesis that heterosis as exhibited in germ weight will be reflected in field yield. However, it should be noted that all of these lines and crosses are much alike because they were derived from a single variety. This conclusion receives slight confirmation from the field yields of the reciprocal crosses (B×D and D×B), where the cross having the heavier germ had a slightly higher yield.

In the hybrids of Pipe and Pawnee, the greater increases in embryo weights relative to those of endosperm suggest that hybrid vigor is not solely the result of the interaction of complementary dominant factors favorable for growth. However, the data in table 5 support the contrary position. In these hybrids, where increases in weight of embryo were obtained, increases of comparable magnitude were found also in the endosperms. The percentage increase or decrease in weight of embryo and endosperm is shown in table 6.

TABLE 6.—*Percentage increase or decrease in weight of the endosperm and embryo of hybrid seed over that of the female parent and of the average of both parents*

Hybrid between inbred lines	Hybrid—female parent		Hybrid—average of both parents	
	Endosperm	Embryo	Endosperm	Embryo
	Percent	*Percent*	*Percent*	*Percent*
A × B	22.4	24.0	53.1	45.7
C × A	−4.6	−36.4	−21.3	−38.2
B × D	−4.4	4.0	−19.5	−5.8
D × B	16.0	16.6	34.4	28.5

The inbred lines are lettered, and it will be noted that hybrids B×D and D×B are reciprocals and that A×B and C×A have one parent in common. It should also be noted that the male parent of hybrid A×B is the female of B×D and the male of D×B.

Hybrids B×D and C×A had germs weighing less than the mean of their parents, and the germs of hybrid C×A actually weighed less than the germs of its female parent. Hybrid B×D does not differ significantly in germ weight from its female parent nor from the mean of both parents. The differences between the other three hybrids and their parents in germ weights may be considered significant.

When tested with a common check, the crosses A×B and B×D both exceeded the check in pounds of grain per plant, the former by 0.14 pound and the latter by 0.13 pound. Both increases are significant, with probabilities of 0.002. The hybrid C×A, on the other hand, was below the check in yield of grain per plant by 0.09 pound. This decrease may also be considered significant, although the probability of its being a chance decrease is 0.03. The order of these three hybrids in their yields of grain follows that of their germ weights.

Hybrid D×B was not tested, but the following year a direct yield comparison was made between the reciprocals B×D and D×B. In this test D×B exceeded B×D in yield by 4 percent, but the yield test could not establish differences of less than 15 percent. The field yields, however, corresponded with the germ weights.

The results of the field tests of these four hybrids may be considered as at least not contradictory of Ashby's findings. In addition, the germ weights of these crosses confirm Sprague (*12*) and Paddick and Sprague (*10*), in that the weights of hybrid germs are not invariably heavier than those of their parents, although the resulting plants show hybrid vigor.

GERM WEIGHTS IN F_2

The early results on the relation of germ weight to subsequent yield stimulated investigation, and as a result of this enthusiasm, a study was made of the germ weights in the second generation of the hybrid A×B; the F_1 data were given in table 5.

In this study an effort was made to sort the seeds visually into germ sizes irrespective of seed weight. Five groups were made because of pronounced differences in seed shape. There were flat medium, flat large, round medium, round large, and small. The flat and round refer to seed shape; large, medium, and small, to germ size. The ear proved to have the distribution shown in table 7.

TABLE 7.—*Distribution of the F_2 seeds of the hybrid A × B based on selection for germ size and seed shape*

Seed character	Seeds	Class	Seed character	Seeds	Class
	Number	*Percent*		*Number*	*Percent*
Flat medium	84	34.3	Small	33	13.5
Flat large	46	18.8			
Round medium	51	20.8	Total	245	100.0
Round large	31	12.6			

Twenty-five seeds from each of these groups were weighed individually for germ size, and the results are shown in table 8.

TABLE 8.—*Mean weight of whole seeds and endosperm and embryo fractions from the F_2 of hybrid A × B*

Seed group	Air-dry weight of entire seed	Oven-dry weight		
		Entire seed	Endosperm	Embryo
	Gram	*Gram*	*Gram*	*Gram*
Flat medium	0.1924±0.0032	0.1774±0.0029	0.1595±0.0027	0.01781±0.00048
Flat large	.2161± .0048	.2009± .0043	.1779± .0038	.02297± .00075
Round medium	.2083± .0082	.1934± .0076	.1737± .0074	.01974± .00064
Round large	.2255± .0071	.2086± .0066	.1866± .0061	.02197± .00100
Small	.1587± .0076	.1462± .0071	.1305± .0065	.01568± .00076

Unquestionably, the selection for germ size was effective, but it will be observed that it was simply an indirect selection for seed size. As a more direct measure of this relation, the correlation between germ weight and the dry weight of seed without the germ has been calculated separately for each of the five seed groups. These five coefficients are shown in the last column of table 9.

TABLE 9.—*Dry weight of germ and correlation with dry weight of seed within five classes in the F_2 of hybrid A × B*

Seed class	Weight of germ [1]	Correlation (*r*) of germ weight with weight of seed without the germ	Seed class	Weight of germ [1]	Correlation (*r*) of germ weight with weight of seed without the germ
	Percent			*Percent*	
Flat medium	9.3	0.38	Round large	9.7	0.43
Flat large	10.6	.59	Small	9.9	.71
Round medium	9.5	.26			

[1] Measured as percentage of air-dry weight of seed.

The subgroup population of only 25 seeds is too small to attach significance to all of these correlations. Combining the five groups into a single population of 125 seeds on the assumption that the selection was not efficient enough to separate the groups, the correlation between seed weight (without the germ) and germ weight is found to be 0.614 with a regression of germ weight on seed weight of 0.083.

It is apparent from table 9 that the relative weight of the germ to the total dry weight (without the germ) does not differ appreciably between the seed groups.

The frequency distribution of germ weight is very irregular, with an ill-defined mode and a mean weight about 10 percent below that of the F_1. The weights ranged from 0.0087 to 0.0324 with a mean of 0.0194. A large part of this unsatisfactory distribution results from the variability of seed weight. When germ weight is corrected for the regression on seed weight, the distribution achieves greater regularity, as shown in figure 1. There is no evidence of bimodality.

In another study, not reported here, germ weights were recorded of Cuzco maize. The mean weight of the oven-dry seeds was 1.149 ±0.021 gm., and the mean germ weight was 0.104±0.003 gm. The germ weight, therefore, was 9.1 percent of the seed weight, which is about the proportion found for most mature seeds.[3]

The correlation of seed weight (without the germ) and germ weight in the Cuzco maize was $r=0.393$ with a regression of germ weight on seed weight of 0.06.

Ashby concluded there was no correlation between seed weight and germ weight because in his material the lines having the heaviest seeds were not the ones with the heaviest germs.

If endosperm weight and germ weight are controlled, at least in part, by the same genic interactions, as would appear to be the case

[3] In immature seeds the dry weight of germs comprises a smaller proportion of the total dry matter. The germs of seed 21 days after fertilization are approximately 6 percent of the total dry weight.

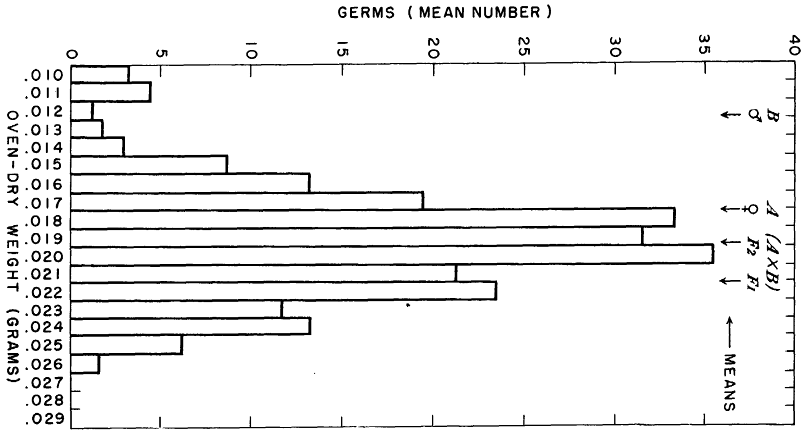

FIGURE 1.—Frequency distribution of germ weight for the seeds of the second generation of the hybrid A × B corrected for the regression of germ weight on seed weight.

from the data shown in table 6, then the weights of these two seed parts would not be independent. It would seem to be a safe inference that when hybrid seeds are heavier than those of their female parent the germs of such seeds will also reflect this greater weight. It is quite likely that interclass correlation would be low where diverse lines comprise the population, and where only a few lines are involved the correlation of seed weight and germ weight might even be negative. In the present examples it is evident that, given a population comprised of a mixture of Cuzco and any of the other sorts considered in this study, the correlation between seed weight and germ weight would be very close indeed.

GERM WEIGHTS IN EARLY STAGES OF GERMINATION

The weight of the germ in the resting stage must be determined by the rate of growth and the period elapsing between fertilization and dormancy. Given germs with equal rates of growth, their final weights must be equal unless their growing periods differ. If the resting stage could be eliminated, as it is in those mutations in maize designated as germinating seeds, or vivipary, the germ weight would not differ in character from seedling or plant weight. It follows that prolonging the growing period of the germ before the resting stage would result in adding to its weight, and since the resting stage can be eliminated it may be argued that when seeds are germinated in the dark, following their dormancy, the rate of increase in seedling weight with time corresponds to that which would have obtained had no resting period intervened. If this holds, then hybrids with small germs in the dormant stage should have slower growth rates in the early stages of germination than hybrids with heavy germs.

As a preliminary test of this point, three lots of seed from two of the F_1 hybrids shown in table 5, namely C×A and D×B, were germinated in the dark under identical conditions, and samples were withdrawn at the end of 24, 48, and 72 hours. From these samples the results given in table 10 were obtained.

TABLE 10.—*Mean oven-dry weight per seed of maize after various periods in a germinating chamber*

[F_1 hybrids (C × A) and (D × B) shown in table 7]

Period	Hybrid C × A			Hybrid D × B		
	Entire seed	Endosperm	Embryo	Entire seed	Endosperm	Embryo
	Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
Resting	0. 1843±0. 0017	0. 1738±0. 0017	0. 01053±0. 00038	0. 2659±0. 0058	0. 2482±0. 0054	0. 01764±0. 00057
24 hours	. 1792± . 0026	. 1618± . 0024	. 01738± . 00011	. 2635± . 0057	. 2384± . 0053	. 02494± . 00051
48 hours	. 1798± . 0024	. 1618± . 0022	. 01810± . 00028	. 2762± . 0061	. 2500± . 0056	. 02619± . 00086
72 hours	. 1827± . 0031	. 1615± . 0027	. 02116± . 00046	. 2628± . 0041	. 2344± . 0037	. 02837± . 00094

Considering the rate of increase in oven-dry weight of embryo, it is seen that C×A had a rate of 1.22 and D×B a rate of 1.17. The rates for the several periods are given in table 11.

TABLE 11.—*Mean rate of increase in dry matter of the germs of germinating seeds of the hybrids C×A and D×B for various periods*

Period	Hybrid C×A	Hybrid D×B
1st 24 hours	1. 65±0. 06	1. 41±0. 05
2d 24 hours	1. 04± . 02	1. 05± . 04
3d 24 hours	1. 17± . 03	1. 08± . 05
Entire period	1. 22± . 10	1. 17± . 07

It is seen that the hybrid C × A, which started with the less capital, had the more rapid rate of increase. The high rate in the first 24 hours accounts for most of this greater rate. If it is assumed that the germs of both hybrids were potentially of the same weight and that the observed difference in dormant weight resulted from an interrupted development prior to maturity in the case of C × A, then the rate of increase of the two hybrid germs might be compared for the periods when their initial weights were identical.

After 24 hours in the germinator, hybrid C × A had germs comparable in weight to those of hybrid D × B at the resting stage. The rate of increase for C × A for the period after the first 24 hours is 1.10.

None of these differences in rate, except that for the first 24-hour period, may be considered as significant.

RATE OF INCREASE IN WEIGHT OF GROWING GERMS

No data were collected showing the rates of growth of the embryos of these hybrids before dormancy. However, field studies have been made on other samples. The determination of rates of weight increase in germs on plants growing under field conditions offers many pitfalls because of the changing environment.

Two sorts of tests have been made with seeds maturing in the field. In one test, sections from ears pollinated at a known time were removed at various periods and rapidly dried. The remaining sections were removed at later periods, and the differences in germ weight between the periods were used to determine the rate of growth. In another set of tests, groups of whole ears pollinated on the same day were harvested at different dates, and the differences in germ size between the early and later periods were used to determine the rate of growth. In both experiments the ears were of the same stock and the pollen used was composited from a number of sister plants.

The first method introduces a possible error if the seeds at the top half of an ear have smaller germs than those at the bottom half. The error from this source may be reduced by using for germ weights the seeds nearest the cut half in each case. However, for reasonable populations of germs, several rings of seeds must be used, so that even under ideal conditions many of the seeds on the separate ear sections are widely separated. Further, it often happens that on the section of the ear left to mature on the plant the seeds near the cut end either fail to attain a dormant stage or else this is of short duration, as many of them germinate before harvest.

The second method, as well as the first, introduces the unknown element of environment, as conditions for germ growth may change so that the observed differences may result, at least partly, from this cause. In the second method also there is introduced the element of genetic variation between plants as well as any differences of environment resulting from the fact that the plants occupy different locations.

The results obtained by these two methods are shown in table 12.

TABLE 12.—*Mean daily rates of increase in germ weights of seeds developing on field-grown plants*

Part of ear and period on which rates were based	Daily rates of increase in germ weights of seeds on—		Part of ear and period on which rates were based	Daily rates of increase in germ weights of seeds on—	
	Ears pollinated July 27	Ears pollinated August 1		Ears pollinated July 27	Ears pollinated August 1
Ear sections:			Whole ears:		
Aug. 21 to 23	1.135±0.039	1.327±0.090	Aug. 23 to 26	1.004±0.029	1.084±0.047
Aug. 23 to 26	1.072± .039	1.064± .021	Aug. 26 to 28	1.162± .025	1.192± .059
Aug. 26 to 28	1.082± .081	1.163± .046			
Aug. 28 to 30	1.056± .011	1.059± .041			

The errors show how unsatisfactory these methods are. There is little agreement between the results obtained on whole ears and on sections where the pollinations were made on July 27, whereas the agreement between these two methods is very good for the pollinations made on August 1. The study would have to be greatly extended before it could be determined whether or not the rate of increase in weight at these early stages was constant.

In conclusion, it may be pointed out that these early rates are of the same order of magnitude as those found for the first 3 days of germination.

GERM WEIGHTS OF VARIOUS SEED TYPES

On the theory that onset of dormancy interrupts the growth of the embryo it was thought possible that where sweet and starchy seeds developed upon the same ears the sweet seeds would have heavier embryos than starchy seeds, because sweet kernels retain a high moisture percentage longer than starchy seeds.

A self-pollinated ear segregating for sweet from a progeny repeatedly backcrossed to starchy until 93.75 percent of the genes were common to both seed types was examined. This ear gave the weights shown in table 13, ear 1.

TABLE 13.—*Mean weight of whole seeds of various types, and endosperm and embryo fractions*

[Seeds of contrasting types borne on same ear]

Ear No. and seed type	Air-dry weight of whole seed	Oven-dry weight of whole seed	Oven-dry weight of endosperm	Oven-dry weight of embryo
Ear 1:	*Gram*	*Gram*	*Gram*	*Gram*
Starchy	0. 3153±0. 0052	0. 2929±0. 0049	0. 2788±0. 0051	0. 01409±0. 00051
Sweet	. 2569± . 0057	. 2389± . 0063	. 2065± . 0068	. 03240± . 00195
Ear 2:				
Starchy	. 4691± . 0085	. 4333± . 0080	. 3888± . 0072	. 04449± . 00128
Sweet	. 3368± . 0099	. 3084± . 0089	. 2728± . 0087	. 03558± . 00120
Ear 3:				
Horny	. 3257± . 0039	. 3045± . 0038	. 2799± . 0036	. 02456± . 00064
Waxy	. 3264± . 0045	. 3040± . 0042	. 2807± . 0043	. 02333± . 00081
Ear 4:				
Horny	. 3533± . 0030	. 3308± . 0030	. 3057± . 0032	. 02505± . 00079
Waxy	. 3377± . 0037	. 3147± . 0034	. 2929± . 0035	. 02181± . 00067
Ear 5:				
Flinty	. 2508± . 0021	. 2374± . 0022	. 2177± . 0022	. 01964± . 00064
Floury	. 2479± . 0023	. 2315± . 0024	. 2094± . 0196	. 02221± . 00066
Ear 6:				
Blue	. 2631± . 0021	. 2409± . 0016	. 2197± . 0021	. 02118± . 00098
Red	. 2678± . 0042	. 2452± . 0038	. 2178± . 0042	. 02740± . 00076
Ear 7:				
Yellow	. 1849± . 0013	. 1726± . 0011	. 1534± . 0011	. 01927± . 00025
White	. 1812± . 0018	. 1684± . 0017	. 1506± . 0018	. 01777± . 00033

The difference in weight of embryo is clearly significant and suggests that the slower maturation of sweet kernels, as indicated by their higher moisture content, may afford the germ a longer growing period before dormancy.

A second ear was examined from a similar but unrelated backcrossing experiment where 87.5 percent of the genes were common to both seed types. In this instance the seeds did not result from self-fertilization but were from crosses between sibs. The weights are given in table 13, ear 2.

In this instance the embryos of sweet seeds are most certainly lighter in weight than those of starchy seeds on the same ear, a result which contradicts the hypothesis of a longer growing period for the embryos of sweet seeds.

A similar study was made with respect to germ weights in horny and waxy seeds on the same ears where because of repeated backcrossing most of the genes were common to both seed types. The weights are given in table 13, ears 3 and 4.

Insofar as these two ears are useful for generalization, it appears that when the female gametes are all waxy and the male is heterozygous (ear 4) the germs of nonwaxy seeds are significantly heavier than those of their waxy sibs. The difference in weight of germ is greater than the corresponding difference in weight of endosperm. When the female is heterozygous (ear 3), the endosperm of the waxy seeds is actually, though not significantly, heavier than that of the horny seeds on the same ear. The germs of the waxy seeds, however, are lighter in weight, though the difference of 5 percent is not significant.

There is no evidence from either of these ears that the waxy type of endosperm provides a longer growing period for the germ before the onset of the resting stage.

A fifth ear was examined, where the contrasting seed characters were flinty-floury. This ear was from an Indian variety inbred for only two generations. The observed weights are given in table 13, ear 5.

This result might be taken as an indication that the physical character of the endosperm affected germ growth. However, two other ears were tested where the differences in seed type were colors and the endosperms were alike in texture.

The first of these is an Indian corn inbred for several generations but segregating for blue and red aleurone (*Pr, pr*). The observed weights are given in table 13, ear 6. In this case the embryo weight of the seeds having the recessive color class exceeds that of the dominant seed group by almost 30 percent.

The second of these color groups involved yellow and white endosperms. The observed weights are given in table 13, ear 7. In this instance the difference in weight of embryo between the two seed classes is also significant, but the recessive seed class has the lightest embryos.

Although there is little likelihood that heterosis is involved, the differences in germ weight between seed classes of the same ear are of the same order as those found between hybrids and their inbred parents and in some instances are of such a magnitude that they should result in wide differences in mature plant size, unless there is a compensating difference in growth rate.

From these data, accumulated for the several seed classes paired on the same ears, it is not possible to formulate conclusions other than that significant differences in germ weight are to be found in various seed groupings, even where most of the gene background is uniform for the classes.

SUMMARY AND CONCLUSIONS

The results reported by Sprague (*12*) are confirmed. Hybrid vigor is often, though not invariably, reflected in the dry weights of the resting embryos of maize. However, in those cases where the hybrid embryos are lighter in weight than those of their parents the plants grown from them exceed the parents in size.

The rate of increase in dry matter following dormancy is greater in hybrids than in their parents in the early stages but the greater rate is not maintained to maturity.

Seed groupings based on endosperm texture and color but with uniform genic backgrounds disclose differences in germ weights.

The increase in weight of embryo relative to the increase in weight of endosperm offers equivocal evidence on the hypothesis that hybrid vigor results from the interaction of complementary dominant factors favorable for growth.

The possibility remains that the measure of heterosis afforded by the germ weight of hybrids, in comparison with that of their parents, can be used as a preliminary evaluation of a series of crosses. The data available at present are inadequate to determine just how useful this criterion may be and, of course, the dry weight of the ultimate plant is only one element governing the choice of hybrids. If germ weights prove of value, the tedious operation of removing dormant germs apparently can be obviated by utilizing the early stages of germination.

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