# THE RELATIONSHIP OF HETEROSIS AND GENETIC DIVERGENCE IN MAIZE[1]

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HETEROSIS in maize appears to increase with increased genetic divergence of the parent populations over a rather wide range of diversity (MOLL, SALHUANA and ROBINSON 1962). It does not necessarily follow, however, that this relationship will hold throughout the entire range of diversity in the species. It is widely accepted that cumulative differences between isolated populations may eventually become great enough to cause genic unbalance in population hybrids, which may be manifest by poor $F_1$ viability. This is considered to be one of the possible steps toward speciation; and inferiority of interspecific hybrids is not uncommon. In the case of an organism which shows heterosis in crosses between divergent populations, it seems logical to expect that in crosses of extremely divergent populations, the expression of heterosis may be limited by unharmonious gene combinations in the $F_1$ hybrid. At higher levels of genetic divergence, then, an increase in divergence might be associated with a decrease in heterotic expression. The issue in this report is whether or not heterosis in maize continues to increase at higher levels of diversity.

## MATERIALS AND METHODS

*Genetic material:* It was decided at the outset that the degree of divergence among the populations to be chosen for this study would be inferred from their probable ancestry together with consideration of geographical separation and adaptation to different environments. Although the choice of varieties with differences in adaptation provides assurance of genetic divergence, these differences pose obvious difficulties in the selection of suitable environments for evaluation. In order to balance effects due to differences in adaptation, it was decided to grow the experiment in each region represented by the populations to be included. This decision, in turn, limited the choice of populations to those which would grow reasonably well and produce grain in widely different environments.

Preliminary tests indicated that the six varieties of the earlier experiment (MOLL *et al.*, 1962) would grow in all three regions represented, as well as in Mexico. Therefore, these varieties were chosen for this study since they not only met the requirements, but would provide an evaluation of the earlier study as well. In order to extend the range of diversity, Zapalote Chico and Zapalote Grânde of southern Mexico seemed to be logical populations to include in the study. Experience has shown them to have a wide range of adaptation. It appears that they are somewhat related to each other (WELLHAUSEN, ROBERTS, HERNANDEZ and MANGELSDORF 1952), and seem to be quite unrelated to the other populations chosen.

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The relative degrees of relationship among the populations in the test are indicated by a survey of their probable ancestry. The varieties of the Southeast, represented by Jarvis and Indian Chief, are descendants of the older southern dents crossed with northeastern or corn belt flints. The old southern dents are considered to have a Mexican origin (ANDERSON and BROWN 1958). The midwestern varieties, represented by Krug Yellow Dent and Reid Yellow Dent, are thought to have arisen from intercrosses of the old southern dents and the northern flints also (BROWN 1953b). The Puerto Rican varieties, Mayorbela and Diente de Caballo, have arisen from descendants of the older Caribbean races with introgression from corns of the United States. The Caribbean races appear to trace to the corns of eastern South America (BROWN 1953a).

Zapalote Chico and Zapalote Grande of Mexico are ancient relative to the other varieties in the test, and may have been developed in preColumbian times. Zapalote Chico may have been derived from the races Nal Tel and Tepecintle, whereas Zapalote Grande is thought to have descended from Zapalote Chico and Tehua (WELLHAUSEN *et al.*, 1952).

Eight levels of genetic divergence were postulated (Table 1) on the basis of ancestral relationships and differences in adaptation. Crosses within the varieties themselves would represent, in essence, no genetic divergence. The next level, with a relatively small degree of divergence, is represented by crosses of varieties from the same region; i.e., Jarvis × Indian Chief, Krug Yellow Dent × Reid Yellow Dent, etc. The third level is represented by crosses of varieties of the Southeast and Midwest, which are presumably related both through the old southern dents and the northern flints and yet a degree of divergence is expected on the grounds of geographical separation. The fourth and fifth levels involve the Puerto Rican varieties crossed with varieties of the United States. Geographical proximity and consideration of environmental factors, such as day length and length of growing season, led to the hypothesis that the Puerto Rican varieties are more similar to those of the Southeast than to those of the Midwest. The greatest levels of divergence are crosses of the United States and Puerto Rican corns with the Mexican corns, Zapalote Chico and Zapalote Grande, which appear to be quite unrelated to the other populations in the study. Since the United States varieties are descendants of corns with Mexican ancestry, it was presumed that these Mexican races might be somewhat more closely related to the United States varieties than to the Puerto Rican varieties. Because of geographical proximity and closer similarity of certain environmental factors, the Mexican corns might be more similar to the southeastern varieties than to the midwestern varieties.

*Experimental procedure:* The experiment comprised the eight populations, the 28 $F_1$ crosses among them and the corresponding 28 $F_2$ populations. Each $F_1$ population was made by random plant-to-plant crosses of a minimum of 100 plants of each of the two parental varieties. Each $F_2$ population was made by random plant-to-plant pollinations of at least 100 plants within an $F_1$ population.

The 64 entries were planted in randomized complete blocks with ten replications in each of

TABLE 1

*Eight levels of genetic divergence in order of increasing divergence*

| Level of divergence | Represented by: (Populations identified by region of adaptation)* |
|---|---|
| I | Within population |
| II | Crosses of populations from same region |
| III | Southeastern U.S. × Midwestern U.S. |
| IV | Southeastern U.S. × Puerto Rico |
| V | Midwestern U.S. × Puerto Rico |
| VI | Southeastern U.S. × Mexico |
| VII | Midwestern U.S. × Mexico |
| VIII | Puerto Rico × Mexico |

* Populations included from each region: Southeastern U.S., Jarvis and Indian Chief; Midwestern U.S., Krug Yellow Dent and Reid Yellow Dent; Puerto Rico, Mayorbela and Diente de Caballo; Mexico, Zapalote Chico and Zapalote Grande.

the four geographical regions represented by varieties in the test. The material was grown at Lewiston, North Carolina; Lincoln, Nebraska; Isabela, Puerto Rico; and Tepalcingo, Mexico. Data were taken on the number of days from planting to tasseling, the average number of ears per plant, and the average yield of ear corn per plant. At North Carolina, Puerto Rico and Mexico, the corn was dried to uniform moisture before weighing. At Nebraska, the moisture content of the corn at harvest was determined, and the field weights adjusted to a common moisture content. Data for number of ears and yield were taken only on competitive plants.

## RESULTS

The data for the three traits are summarized in Table 2 by the levels of diversity postulated. The means for level I are averages of the eight varieties, and means for levels II through VIII are averages of four variety crosses over all four geographical regions of the study. $F_1$ and $F_2$ generations exhibited similar patterns for all three traits. Yield tended to increase with increasing divergence of the parents up to levels IV and V, which include the crosses of United States and Puerto Rican varieties. Further increases in genetic divergence resulted in decreased yield. Ear number and days to tassel show little or no association with either levels of diversity or yield.

Since the values given for each level are averages of several entries, the relative amount of variation between and within levels is pertinent. Variation for yield among levels was found, by analysis of variance, to be seven to nine times greater than variation within levels, and the corresponding factors for ear number and days to tassel are approximately two and seven, respectively.

Heterosis, expressed as the difference between the $F_1$ mean and the average of the parental varieties (Table 3), also increases with increased divergence of the parents up to levels IV and V, and then decreases with further increases in divergence. (Note that heterosis for days to tassel is in the direction of earliness.) The same pattern is seen in the difference between the $F_1$ and $F_2$ means for yield but not for the other two traits.

TABLE 2

*Average performance for three traits summarized by level of divergence of parental populations*

| | Trait | | | | | |
|---|---|---|---|---|---|---|
| | Yield* | | Number of ears | | Days to tassel | |
| Level of divergence | $F_1$ | $F_2$ | $F_1$ | $F_2$ | $F_1$ | $F_2$ |
| I | .352† | ... | 1.21† | ... | 72.5† | . |
| II | .392 | .359 | 1.26 | 1.20 | 72.0 | 71.8 |
| III | .488 | .456 | 1.20 | 1.22 | 70.4 | 71.2 |
| IV | .520 | .454 | 1.38 | 1.34 | 76.3 | 76.7 |
| V | .520 | .456 | 1.21 | 1.22 | 72.2 | 72.1 |
| VI | .405 | .372 | 1.32 | 1.35 | 69.4 | 69.6 |
| VII | .380 | .355 | 1.16 | 1.16 | 66.4 | 66.1 |
| VIII | .323 | .313 | 1.26 | 1.22 | 73.2 | 72.9 |

* Pounds per plant.
† Mean of the varieties.

TABLE 3

*The average differences between the* $F_1$ *and midparent* (MP) and between* $F_1$ *and* $F_2$ *generations for three traits, and the difference between the* $F_1$ *and the high parent (HP) for yield for each level of genetic divergence*

| Level of divergence | Yield† | | | Number of ears | | Days to tassel | |
|---|---|---|---|---|---|---|---|
| | $F_1$-MP | $F_1$-$F_2$ | $F_1$-HP | $F_1$-MP | $F_1$-$F_2$ | $F_1$-MP | $F_1$-$F_2$ |
| II | .040 | .033 | .023 | .049 | .055 | —0.5 | 0.2 |
| III | .076 | .032 | .063 | .008 | —.027 | —1.4 | —0.8 |
| IV | .134 | .066 | .102 | .111 | .037 | —1.1 | —0.4 |
| V | .140 | .064 | .114 | .070 | —.012 | —1.7 | 0.1 |
| VI | .081 | .033 | —.013 | .042 | —.024 | —1.7 | —0.2 |
| VII | .062 | .025 | —.026 | .000 | —.002 | —1.2 | 0.2 |
| VIII | .031 | .010 | —.031 | .035 | .041 | 0.0 | 0.3 |

* Average of the parental populations.
† Pounds per plant.

The yield of all $F_1$ populations exceeded the average of the parents even in crosses of the most divergent populations. On the other hand, the comparison of $F_1$ yield with that of the high parent shows that the $F_1$'s representing the three highest levels of divergence were inferior to the high parent.

The relationship of heterosis for yield to level of divergence appears to be remarkably consistent among the four test locations (Table 4). Although the magnitude of differences among levels varies (largest differences at Nebraska; smallest differences at Mexico), the pattern is essentially the same at all four locations. The same is true for differences between $F_1$ and $F_2$ means, although the differences among levels are smaller and thus the patterns are less distinct.

TABLE 4

*The average differences in yield* between the* $F_1$ *and midparent† (MP) and between* $F_1$ *and* $F_2$ *generations at each of the four test locations*

| Level of divergence | North Carolina | | Nebraska | | Puerto Rico | | Mexico | |
|---|---|---|---|---|---|---|---|---|
| | $F_1$-MP | $F_1$-$F_2$ | $F_1$-MP | $F_1F_2$ | $F_1$-MP | $F_1$-$F_2$ | $F_1$-MP | $F_1$-$F_2$ |
| II | .029 | .037 | .075 | .036 | .024 | .039 | .031 | .015 |
| III | .059 | .017 | .182 | .075 | .029 | .016 | .052 | .040 |
| IV | .163 | .050 | .182 | .110 | .073 | .040 | .116 | .064 |
| V | .181 | .082 | .236 | .095 | .053 | .037 | .088 | .043 |
| VI | .056 | .012 | .111 | .035 | .062 | .031 | .097 | .029 |
| VII | .080 | .025 | .076 | .043 | .052 | .022 | .038 | .006 |
| VIII | .028 | .023 | .036 | .006 | .028 | .016 | .035 | .027 |

* Pounds per plant.
† Average of parental populations.

## DISCUSSION

As a result of some of the earlier work with maize (EAST and HAYES 1912; EAST 1936; HAYES and JOHNSON 1939; JOHNSON and HAYES 1940; WU 1939), it has become commonly accepted that crosses of more distantly related parents show greater heterosis than crosses of more closely related parents. This conclusion was based upon a rather restricted range of genetic divergence, and may not hold over the entire range of divergence encountered in the species. For instance, RICHEY (1922) reviewed evidence which suggested that genetic divergence as reflected in differences in endosperm types of the parents was related to $F_1$ hybrid performance. Recent work by PATERNIANI and LONNQUIST (1963), which involved crosses of widely divergent races, showed little or no average difference in heterosis of race crosses which involve the same endosperm type versus those which involve different endosperm types.

The populations chosen for this study were representatives of groups of maize populations distinct enough in origin that the relative rank of genetic divergence among them would be reasonably certain. The relative rank of diversity of the various kinds of crosses was postulated *a priori*. On this basis, the results are interpreted as evidence that there is an optimum degree of genetic divergence for maximum expression of heterosis in maize. Furthermore, this optimum occurs within a range of divergence that is narrow enough so that incompatibility barriers, such as those caused by cytological irregularities, are not apparent.

It must be recognized that the evidence is based upon a rather restricted sample of varieties. The decline in heterosis which is apparently associated with higher levels of genetic diversity involves crosses which have either Zapalote Grande or Zapalote Chico as one of the parents. Although these two populations are different enough to be classed as separate races by some authorities (WELLHAUSEN *et al.*, 1952), they have many phenotypic similarities. Both of these populations are relatively low in yield of grain, even in Mexico under conditions similar to those where they are commonly grown. This raises the possibility that the lower heterosis observed in crosses involving these races is associated with their lower yield rather than with greater genetic divergence. Results obtained by PATERNIANI and LONNQUIST (1963) show that the $F_1$ performance of high × high crosses (with respect to grain yield) was higher than that of high × low or low × low crosses. However, heterosis (as percent of the parental mean) shows exactly the reverse pattern; i.e., high × high crosses gave the lowest heterosis and low × low the greatest. The data presented here display the same pattern (with respect to the levels of genetic divergence postulated) whether the data are expressed as average $F_1$ performance, or as heterosis expressed as either the deviation from the parental mean or as the deviation from the high parent. The fact that the pattern is consistent is interpreted as supporting evidence that the levels of heterosis observed are associated with the levels of genetic divergence.

The populations representing varieties from the Southeast, the Midwest, and Puerto Rico together with the $F_1$ hybrid populations among them, were the same as those included in a study by MOLL *et al.*, 1962. However, the earlier study had been grown in only one region and possible effects due to differences in adaptation could not be evaluated. The results here show that the relative rank of heterosis for diversity levels averaged over the four regions is in complete agreement with the results of the previous study. The relative magnitude of expression of heterosis varied from region to region, and although the rank of heterosis values of the various levels is not exactly the same from region to region, the discrepancies that exist involve small differences in magnitude and may be due to experimental errors rather than to differences in adaptation.

## SUMMARY

Two populations from each of four geographical regions—southeastern United States, Midwestern United States, Puerto Rico, and southern Mexico—were intercrossed in all combinations, and the resulting $F_1$ populations were advanced one generation to give $F_2$ populations. The degree of genetic diversity among the parental populations was inferred from a survey of ancestral relationships and geographical separation with adaptation to different environments. The parental, $F_1$ and $F_2$ populations were grown in the four regions of adaptation in order to study the relationship of heterosis to genetic divergence. The results indicate that heterosis increased with increased divergence within a restricted range of divergence, but extremely divergent crosses resulted in a decrease in heterosis.

## LITERATURE CITED

ANDERSON, E., and W. L. BROWN, 1958 The southern dent corns. Ann. Missouri Botan. Garden **35**: 255–268.

BROWN, W. L., 1953a Maize of the West Indies. Trop. Agr. **30**: 141–170. —— 1953b Sources of germ plasm for hybrid corn. Proc. 8th Annual Hybrid Corn Industry-Research Conference. **8**: 11–16.

EAST, E. M., 1936 Heterosis. Genetics **21**: 375–397.

EAST, E. M., and H. K. HAYES, 1912 Heterozygosis in evolution and plant breeding. USDA Bur. Plant Ind. Bull. **243**: 1–58.

HAYES, H. K., and I. J. JOHNSON, 1939 The breeding of improved selfed lines of corn. J. Am. Soc. Agron. **31**: 710–724.

JOHNSON, I. J., and H. K. HAYES, 1940 The value in hybrid combinations of inbred lines of corn selected from single crosses by the pedigree method of breeding. J. Am. Soc. Agron. **32**: 479–485.

MOLL, R. H., W. S. SALHUANA, and H. F. ROBINSON, 1962 Heterosis and genetic diversity in variety crosses of maize. Crop. Sci. **2**: 197–198.

PATERNIANI, E., and J. H. LONNQUIST, 1963 Heterosis in interracial crosses of corn (*Zea mays* L.). Crop Sci. **3**: 504–507.

RICHEY, F. D., 1922 The experimental basis for the present status of corn breeding. J. Am. Soc. Agron. **14**: 1–17.

WELLHAUSEN, E. J., L. M. ROBERTS, E. X. HERNANDEZ, and P. C. MANGELSDORF, 1952 *Races of Maize in Mexico.* The Bussey Inst., Harvard University, Cambridge, Mass.

WU, S. K., 1939 The relationship between the origin of selfed lines of corn and their value in hybrid combination. J. Am. Soc. Agron. **31**: 131–140.