THE RELATIONSHIP OF HETEROZYGOSITY TO HOMEOSTASIS IN MAIZE HYBRIDS¹

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BOTH likeness and constancy are implied by the word homeostasis. In the genetic context, genotypes which interact with a variable environment in such a way as to maintain relative uniformity of an observable characteristic therein fulfill both the spirit and the letter of the concept, homeostasis. Though primarily morphological characteristics have been studied, their stability of development in a variable environment implies physiological adjustments. The capacity for such adjustments is a property of the homeostatic genotype.

DEMPSTER (1956), commenting on LEWONTIN's (1956) use of the term homeostasis, and citing CANNON's original definition, states "It seems clear . . . that homeostasis applies to particular conditions that are maintained in a relatively stable state by special mechanisms that have evolved through natural selection; whereas the causative conditions, which are themselves variable, are called homeostatic devices or mechanisms. Homeostatic conditions seem important aids to, or components of, fitness, but not fitness itself."

GRAFIUS (1956) has studied a theoretical model involving this concept and presented experimental data in its support. Grain yield in oats (*Avena sativa* L.) was represented geometrically as a rectangular parallelepiped of volume W and with edges X (number of panicles per unit area), Y (average number of kernels per panicle), and Z (average kernel weight); thus $X \cdot Y \cdot Z = W$. A universal variety (the homeostatic genotype) was defined as a variety which equals or exceeds the mean yield at all locations in a region. On the basis of the theory developed, among other properties of the universal variety, *changes in X , Y , and Z would tend to counterbalance*; and the edge most subject to change would be the longest, the edge least subject to change, the shortest. GRAFIUS concluded from this analysis that grain yield of some genotypes may remain relatively invariant over a range of environments even as the components of yield vary. The changes in the components are compensating such that their product ($X \cdot Y \cdot Z$) is relatively invariable. The components of yield are homeostatic devices, in the sense of CANNON (DEMPSTER 1956), which assure homeostasis of yield. Homeostasis can be measured in terms of relative variability of a characteristic over a range of environments.

In maize, single cross hybrids are generally more homeostatic for yield and other adaptive traits than are the parental inbred lines (SHANK and ADAMS 1959).

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Two explanations have been advanced to account for superior homeostasis or buffering in heterozygous individuals (THODAY 1955). The first attributes a special property to heterozygosity that promotes versatility in development beyond that possible in homozygotes. The second view attributes no special property to heterozygosity except the classic one of promoting segregation, which leads to superior buffering in hybrids by virtue of the heterozygous balance achieved in outbreeding species by natural selection.

Results bearing on this problem are presented from an experiment on environmental variability of maize hybrids which were produced so as to possess different levels of heterozygosity.

MATERIALS AND METHODS

Random plants of two locally grown open pollinated strains of maize, ELLIS and ARMSTRONG, were initially self-pollinated in 1949, and individual plants selfed each year thereafter through five successive generations to establish a group of nearly homozygous inbred lines. Related lines were produced during the inbreeding phase by selfing sibling plants. Depending upon the stage of inbreeding when sublines were started, some lines were closely related, others were related to a lesser extent, and some were related only through having descended from a common noninbred plant in the open pollinated source strain. From this material it was possible, in 1955, to recombine in single crosses inbred lines possessing a high level of homozygosity within lines, but of varying degrees of relationship between lines. On this account the proportion of heterozygous loci to be expected in the hybrids could be expressed as a function of the calculated inbreeding coefficients of the hybrids themselves.

The hybrids were arrayed in a total of eight groups, differing in level of heterozygosity. Hybrids in groups 1 through 5 were produced with expected inbreeding coefficients (F) of 0.969, 0.923, 0.861, 0.738, and 0.492, respectively. It was assumed that heterozygosity in the hybrids would be proportional to F from the relation $H = 1 - F$. Implicit in the assumption are that (1) no effects result from differential genetic homozygosity of the basic populations, and (2) no natural or artificial selection favoring heterozygosis or particular genotypes occurs during the inbreeding process. In terms of level of heterozygosity, calculated as $H = 1 - F$, the first five groups of hybrids had expected values of 0.031, 0.077, 0.139, 0.262, and 0.508, respectively. Group 6 was composed of intravarietal hybrids from inbred lines related only in having been derived from different open pollinated plants of a particular source variety. Group 7 was made up of intervarietal hybrids, the two inbreds in each case being derived from different source varieties. The hybrids in group 8 were formed from Corn Belt inbreds of diverse origins.

Since the expected level of heterozygosity (H) is based on the inbreeding coefficient, and is therefore relative to the open pollinated source variety as a base, the intravarietal crosses of group 6 were considered to have $F = 0$, or $H = 1$. However, it is reasonably to be expected that the intervarietal crosses of group 7

should be more heterozygous than those of group 6; whereas the level of heterozygosity of crosses in group 8 is probably the highest of all groups. The assignment of relative values of expected level of heterozygosity to groups 7 and 8 is of necessity somewhat arbitrary, though there is sufficient reason for believing them to be arrayed in the correct order of magnitude.

It is recognized that after five generations of selfing complete homozygosity will not have been attained in a diploid organism. The approximately three percent residual heterozygosity in the inbred parents presumably would manifest itself as genetic variance in the hybrid. But this effect would be common to hybrids in groups 1 through 7, and thus could not influence comparisons involving these groups, unless it happened that one or more genes segregating in an otherwise nonsegregating background generated variance as some function of the level of heterozygosity of the rest of the genotype. Group 8 would be less subject to the foregoing qualification. With the acknowledgment of possible trivial bias from this source, it should be clear that in each F_1 all full sib plants were essentially genetically identical, and differences among them could be attributed almost wholly to environmental causes.

The material was grown in 1956 at Brookings on Vienna loam, a relatively uniform upland glacial till soil, and at Menno on a soil complex of variable zonal and solonetz phases derived from glacial till. In 1957 the test was grown again at Brookings, and at Highmore on a variable loam-clay complex derived from glacial till.

In 1956 there were 34 hybrids, and in 1957, 40 hybrids in the field trials. In all locations the plots were single rows 30 hills in length with plants spaced 14 inches apart in the row. Each hill contained a single plant, and each test row was grown between two rows of an adapted single cross hybrid to provide uniform competition throughout the test. Measurements were not taken on plants located at the ends of the row or on plants adjacent to missing hills; otherwise the measured plants were taken successively in the row. Ten plants per plot were measured in the field, the traits being plant height, flag leaf height, and ear node height. At maturity, but before loss of leaves, the ten plants previously measured were cut close to the ground, chopped into short lengths, individually bagged, dried, and weighed. The ear from each plant was then husked and weighed separately.

Coefficients of variation were determined on each plot for each trait. Means of plots were compared by analysis of variance, and the relationship of group means over years and locations to expected level of heterozygosity was evaluated by regression. The levels of heterozygosity calculated for groups 1 through 6 followed a geometric progression so the values of H for all groups were transformed to logarithms for purposes of determining the regression of buffering capacity on heterozygosity.

EXPERIMENTAL RESULTS

Average values of the coefficients of variability for each level-of-heterozygosity group for each character in each environment are presented in Table 1. In each

TABLE 1

*Mean values of coefficient of variability for each level-of-heterozygosity group for each trait in each year and locality**

Trait	Heterozygosity group number							
	1	2	3	4	5	6	7	8
Plant height								
Brookings 1956	7.62	6.65	7.12	5.67	5.01	5.03	4.81	4.69
Brookings 1957	7.47	6.73	6.95	5.90	6.66	4.90	6.71	6.67
Menno 1956	10.44	9.73	8.68	9.54	9.43	6.68	6.98	7.72
Highmore 1957	11.21	9.81	9.20	8.89	8.89	5.75	6.12	6.95
Flag leaf height								
Brookings 1956	8.82	7.99	8.64	6.97	5.68	6.13	5.90	5.52
Brookings 1957	8.67	7.70	8.23	7.10	7.70	5.99	7.80	8.55
Menno 1956	12.28	11.83	10.43	12.22	11.43	8.40	8.36	8.75
Highmore 1957	13.27	11.47	10.59	9.94	9.97	6.66	7.01	7.11
Ear node height								
Brookings 1956	14.53	12.91	16.32	14.16	9.85	9.65	11.03	6.03
Brookings 1957	18.67	11.97	13.68	11.71	10.01	10.20	9.84	11.42
Menno 1956	16.85	15.52	18.74	17.09	13.63	12.10	13.74	10.93
Highmore 1957	20.14	18.50	17.72	17.49	18.15	12.41	11.71	11.20
Total plant weight								
Brookings 1956	19.94	21.48	19.65	19.33	16.20	17.07	16.89	12.05
Brookings 1957	25.22	23.53	18.98	21.93	19.57	20.43	21.67	17.01
Menno 1956	30.01	25.81	23.50	30.21	21.27	23.40	24.46	18.94
Highmore 1957	29.77	27.52	25.01	25.22	20.51	22.69	21.28	14.67
Ear weight								
Brookings 1956	32.87	30.56	25.05	26.62	18.44	15.42	18.10	14.40
Brookings 1957	41.77	38.81	33.65	40.59	25.06	22.49	26.68	19.41
Menno 1956†								
Highmore 1957	36.97	38.59	33.68	37.35	18.18	17.58	20.87	14.96

* 1956: Group 1 included six hybrids; groups 2 through 8 included four hybrids per group.

1957: Groups 1 through 8 included five hybrids per group.

† Data not usable on account of the predominance of barren plants throughout the experiment.

environment and for all traits but one, the heterozygosity groups differed significantly in buffering capacity, the single exception being flag leaf height at Brookings in 1957. In most instances hybrids within levels of heterozygosity also varied significantly in buffering ability (Table 2).

In Table 3 are given the mean coefficients of variation, averaged over all levels of heterozygosity, for each trait, year, and location. These indicate that variability was generally least at Brookings, which was characterized by a more uniform soil and favorable climatic environment, with one notable exception, ear weight in 1957.

The mean coefficient of variation of each group, for each trait in turn, was plotted against the logarithm of the calculated level of heterozygosity for that group (Figure 1). The relationship in each case obviously is essentially linear, and this is conclusively demonstrated in Table 4. From 75 to nearly 90 percent of the variation in buffering capacity can be attributed to the level of heterozygosity of the groups.

TABLE 2

Significance of differences in buffering capacity between and within groups of maize hybrids

Trait	Brookings		Menno 1956	Highmore 1957
	1956	1957		
Plant height				
Between groups	***	*	***	***
Within groups	***	***	N.S.	N.S.
Flag leaf height				
Between groups	***	N.S.	***	***
Within groups	***	*	N.S.	N.S.
Ear node height				
Between groups	***	***	***	***
Within groups	***	***	***	N.S.
Total plant weight				
Between groups	***	***	***	***
Within groups	***	***	**	*
Ear weight				
Between groups	***	***	†	***
Within groups	N.S.	*	†	***

* $P \leq 0.05$.** $P \leq 0.01$.*** $P \leq 0.005$.

† No data obtained.

TABLE 3

Mean coefficients of variation for locations and years, averaged over all levels of heterozygosity, for each trait

Trait	1956		1957	
	Brookings	Menno	Brookings	Highmore
Plant height	5.94	8.75	6.50	8.35
Flag leaf height	7.07	10.57	7.72	9.50
Ear node height	11.97	14.94	12.19	15.92
Total plant weight	17.95	25.01	21.04	23.33
Ear weight	22.68	†	31.06	27.27

† No data obtained.

TABLE 4

Linear regression of coefficient of variability on log heterozygosity for each trait

Trait	Linear regression coefficient	Percent of variability due to regression on heterozygosity
Plant height	— 1.48**	80.267
Flag leaf height	— 1.79**	80.538
Ear node height	— 3.58**	88.296
Total plant weight	— 4.02**	75.492
Ear weight	— 10.62**	81.606

** $P \leq 0.01$.

FIGURE 1.—The relationship of coefficients of variability to log heterozygosity in eight groups of hybrid maize. Groups, as plotted on the abscissa (3-cycle log scale), are given in order of increasing heterozygosity. Variability, plotted on the ordinate, is inversely proportional to homeostasis.

Taken collectively, these data show highly significant linear relationships existing between the level of buffering and the degree of genetic heterozygosity, with high levels of buffering being associated with high levels of heterozygosity. The traits related to length, such as plant-, flag leaf-, and ear node-height, that developed during the earlier more favorable part of the growing season show small regression values and less fluctuation from one level of heterozygosity to another.

DISCUSSION

There was obtained in this experiment compelling evidence of a major dependence of buffering capacity on level of heterozygosity of the groups of maize

hybrids studied. The evidence does not specify whether it is genes for height and weight that must be heterozygous to confer homeostasis, or whether the heterozygosity could refer to "background" genes not involved directly in the immediate events leading to height and weight traits.

In either case the evidence is in accord with the hypothesis that the increased biochemical versatility assumed to be possessed by plants by virtue of the heterozygous state of certain of their genes confers on such genotypes superior homeostasis.

But it has been observed in the present experiment and previously (SHANK and ADAMS 1959) that hybrids belonging to the same level-of-heterozygosity group frequently manifest significantly different buffering properties. These results clearly indicate that heterozygosity *per se* is not the only hypothesis required to account for homeostasis.

LEWIS (1954) supported the idea that biochemical versatility would be manifest, at least in part, in alternative pathways of biosynthesis functional under different environmental optima. Similarly, HALDANE (1954) advanced the possibility that a complex enzyme (presumably gene controlled) whose constituent parts possessed sharp optima for environmental variables would be of definite advantage in an organism subjected to a wide range of environments. Such an enzyme might result from the action of one or more genes in the heterozygous state, or from the simultaneous presence of two or more interacting alleles at different loci; and it is thus a potential property of both heterozygotes and homozygotes. But because they possess a greater number of different alleles, and hence a greatly enhanced frequency of potential allelic and nonallelic interactions, heterozygotes in maize are likely to be more highly buffered than are homozygotes. Differential buffering in homozygotes would depend upon their possessing, in greater and lesser degrees, genetically fixed nonallelic interactions (epistasis).

In a gene pool of a normally outbreeding species the organization of genes may be relationally balanced or coadapted (MATHER 1953; DOBZHANSKY and WALLACE 1953). If lines are extracted from this kind of population, made homozygous, and hybridized so as to produce single crosses of predetermined levels of heterozygosity, it might be expected that buffering capacity of the hybrids would increase proportionately with increasing heterozygosity to the level of the random mating population in approximate equilibrium, but thereafter decrease with further increases in heterozygosity.

A priori it might be assumed that the random mating population in approximate equilibrium is represented most closely in maize by the locally adapted open pollinated variety. If that were true, the intravarietal F_1 's of group 6 on the average should be expected to restore the level of heterozygous balance which had been achieved by natural selection in that population before inbreeding was commenced. The intervarietal hybrids of groups 7 and 8, however, should be less well buffered because the fine adjustments leading to heterozygous balance in a common gene pool had not been achieved, owing to the recent reproductive isolation of the parental source populations.

Any interpretation to be made at this point must rest on the validity of the *a priori* assumption stated above. Since the work of BEAL at Michigan, corn breeders have known that crosses between open pollinated varieties might be heterotic for yield. In a recent experiment involving six North Carolina open pollinated varieties crossed in all possible combinations the average increase of crosses over the midparent amounted to 19.9 percent (ROBINSON *et al.* 1956). The genetic differences between varieties were attributable either to inbreeding due to finite population sizes or to divergent mutational changes in noninterbreeding populations. The authors considered it entirely plausible that the varieties comprised partially inbred populations. This was in agreement with the viewpoint of SPRAGUE (1955), referring to the status of open pollinated varieties in general. They also questioned whether the open pollinated variety is in equilibrium at all.

The evidence, though not conclusive, fails to uphold the assumption that the partially isolated, locally grown, open pollinated variety of maize is the random mating population in approximate equilibrium demanded by the heterozygous balance hypothesis. Rather, it is to be assumed that the recent reproductive isolation has been insufficient either in time or space, to bring about new heterozygous balance, and that variety crosses tend to restore the balance previously achieved by natural selection in the larger population.

The present experiment, then, is concerned in groups 7 and 8 with crosses representing that larger population, that is, with crosses derived from inbred lines out of numerous partially isolated and partially inbred open pollinated varieties, which more nearly tend to restore the state of natural heterozygous balance. On this view, the regression analysis, in accounting for 75 to 88 percent of the variation in buffering by dependency upon heterozygosity, is in satisfactory agreement with the heterozygous balance concept.

By analogy with the geometric representation of a complex trait (GRAFIUS 1956), these results may also be interpreted to suggest that there exist back of each trait studied component processes or characteristics which, if experimentally observed, would be found to vary with fluctuating environment in such a way as to maintain relative constancy of the initial complex trait. If this were true it would be meaningless to discuss the heterozygosity of genes for the complex trait itself, for such genes may not in fact exist. The genes whose biochemical versatility has been inferred may function primarily to adjust the component processes in a compensating fashion. These processes correspond to the special mechanisms or homeostatic devices of DEMPSTER (1956) which are themselves variable but which thereby insure relative stability of particular conditions like height or weight.

It can be suggested further that these processes shift in a compensating fashion because of natural selection for stability of the complex trait, and that heterozygosity is one means of conferring the needed flexibility upon the component processes.

The dependency of homeostasis on heterozygosity in maize hybrids is paralleled

by the relationship of heterosis to heterozygosity. The components hypothesis discussed in this paper as an alternative explanation of homeostasis has previously been proposed as the basis of a new definition of heterosis (GRAFIUS 1956). It was recently shown (GRAFIUS 1959) that 50 percent of the heterosis exhibited in barley hybrids could be accounted for by interactions of three components of grain yield. On this hypothesis, both phenomena, homeostasis and heterosis, result from one fundamental geometry of enumeration and measurement. The genetic system controlling variation of the component processes, however, need involve only the classic concepts of additivity, dominance, and epistasis.

SUMMARY

Homeostasis was measured in eight groups of maize hybrids which had been produced so as to achieve greatly different levels of genetic heterozygosity between groups. Thirty-four hybrids in 1956 and 40 hybrids in 1957 were evaluated at two locations during each year for homeostasis in five traits by comparison of intraplot coefficients of variation.

Evidence of two kinds was obtained. First, homeostasis in these maize hybrids was highly related to the expected levels of heterozygosity of the hybrid groups. For the five traits studied, namely, plant height, flag leaf height, ear node height, total plant weight and ear weight respectively, 80.3, 86.5, 88.3, 75.5, and 81.6 percent of the differences in buffering capacity were attributable to differences in heterozygosity of the eight groups of hybrids. Second, differences between hybrids within groups were, in most cases, highly significant, which suggests that intrinsic heterozygosity is not a sufficient explanation of homeostasis in maize.

Any proposals aimed at explaining homeostasis must account both for the paramount role of heterozygosity, and for the differences in buffering manifested by inbreds, and by hybrids at the same level of heterozygosity.

Three hypotheses are discussed; they are not necessarily mutually exclusive. The first one postulates either the existence of alternative pathways of biosynthesis, optimally functional under different environmental conditions, and depending on heterozygous loci or nonallelic gene interactions, or the presence of a complex enzyme whose constituent parts function alternatively in a fluctuating environment. In theory this proposal seems feasible, but in practice it presents formidable difficulties in the designing of experiments affording a critical test. The data obtained in the current study are consistent with it but are not critical so far as this hypothesis is concerned.

The heterozygous balance hypothesis, with certain assumptions, leads to the prediction that homeostasis should increase with increasing heterozygosity, at least up to the level of heterozygosity established in the random mating equilibrium population. To a satisfactory degree the prediction was fulfilled by the data of this study. The hypothesis, however, fails to account readily for evidence of the second kind, namely, differences in buffering among inbreds or among hybrids at the same level of heterozygosity.

In the third hypothesis it is proposed that back of each major homeostatic trait

are to be found component characteristics or processes which vary in a compensating pattern to promote relative constancy of the complex trait. The flexibility of the components is derived from the biochemical versatility conferred by heterozygosity. But nonallelic gene interactions are capable of conferring differential versatility on homozygotes, and so both kinds of evidence can be answered in this hypothesis. A direct test of this hypothesis was not sought in the present study, but it may be readily tested.

Comparing the first and third hypotheses, it appears that in either case heterozygosity promotes internal adjustment to gain external stability. It is not clear, however, whether adjustments are made at the genic level or at the level of component variation.

The relationship of homeostasis to heterozygosity is analogous to the relationship of heterosis to heterozygosity and, on the components hypothesis, both phenomena might result from a common fundamental geometry of enumeration and measurement.

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