NATURAL MUTATIONS IN INBRED LINES OF MAIZE AND THEIR HETEROTIC EFFECT. I. COMPARISON OF PARENT, MUTANT AND THEIR F_1 HYBRID IN A HIGHLY INBRED BACKGROUND[1]

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IT has been known for over 50 years that inbreeding reduces vigor of the offspring and crossing inbreds restores normal vigor. Frequently the F_1 exceeded the better of the two original parents. Utilization of this increase in vigor was the basis for production of varietal hybrids in corn, and later the development of corn hybrids from inbred lines. Principles used in hybrid corn spread to other crops such as sugar beets, melons and onions, and to swine, poultry and other animals. In view of the economic importance of heterosis, it is surprising that so few critical experiments have been conducted to establish the genetic mechanism involved. It appears probable that further advances in commercial utilization of heterosis may well depend upon further clarification of the types of gene action involved.

SHULL (1914) coined the term "heterosis" to replace the lengthy expression "stimulus of heterozygosity" and also to provide a term which carried no implication as to genetic mechanism involved. Even before the term heterosis was proposed, studies had been reported which were designed to explain the phenomena. The explanations proposed fell into two rather distinct categories. Without attempting to follow the changes in terminology or assumptions as to mechanisms involved, two opposing theories still persist and are commonly designated by the simple terms dominance and overdominance. The former assumes that the combination of dominant or partially dominant favorable growth factors bring about hybrid vigor, while the latter rests on the assumption that the heterozygous state *per se* results in some kind of physiological stimulus expressed as hybrid vigor. In simple genetical symbols the dominance hypothesis assumes that $AA \geqslant Aa$ and the overdominance hypothesis that $Aa > AA$ where A is the dominant, and a the recessive gene. The smallest unit in which overdominance can be studied is the single gene. A gene would show overdominance or a locus would be heterotic if the heterozygote exceeded either homozygote.

Long time inbred lines of maize are assumed to be highly homozygous. If a mutation in such a line occurred at a single locus, it would be possible to

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produce and compare the three possible genotypes *AA*, *Aa* and *aa* in an identical genetic background. Superiority of the *Aa* plants could be attributed to heterotic effects of the single gene under consideration or to other sources of heterozygosity not under direct control. It seems rather important to investigate whether the basic assumption of a one-gene difference holds or whether other heterozygous loci are involved. A crude way to accomplish this would be to isolate one or both of the homozygous genotypes by selfing the heterozygote and to compare the recovered homozygous line with the corresponding parental line, which had not gone through a cross generation since the time the mutation was discovered and isolated.

Numerous older reports in which the heterozygote was superior in some attributes to either homozygote were brought to attention by advocates of the overdominance hypothesis. In the past 30 years, a rather large number of papers have been published on overdominance at a single locus. In most of these studies, the genes under study were not in a homozygous background as e.g. in Drosophila and other animals. In plants, QUINBY and KARPER (1946) compared lines of sorghum which were presumed to differ only in a number of genes for maturity. It was found that plants heterozygous for one of the maturity genes were larger than homozygous plants and this difference was ascribed to one locus. In a previous paper by the same authors in 1945, where the derivation of the lines used in the heterosis study was described, it is apparent that the lines were not isogenic but were segregates obtained after crossing different strains of milo.

HAGBERG (1953) presented an analysis of several cases of monofactorial heterosis in a series of so-called "erectoides" (dense ear) mutants in barley. The mutations were X-ray induced. Overdominance on a single locus was found in two-gene combinations for total plant weight, yield of grain per plant and tillering, one of them also in number of florets per plant.

Natural mutations affecting chlorophyll synthesis, which had occurred in the pure line Golden Barley, were studied by GUSTAFSSON (1946, 1947). The heterozygotes were reported to be superior in one or more of the characters measured, although no data were presented to indicate the significance of the results. When two loci were heterozygous, the heterotic effect seemed to be cumulative and differences in many attributes were significantly in favor of the heterozygous class.

SINGLETON (1943) crossed a semi-dwarf mutant with the inbred line of corn in which the mutation had occurred as well as to an unrelated inbred line. In the former cross the genotype heterozygous for the semi-dwarf locus significantly exceeded the parent line in rate of growth and ear weight. In test crosses to an unrelated source, the cross involving the recessive semi-dwarf gene gave a significant increase over the cross carrying both dominant alleles.

A number of heritable morphological alterations in corn, presumably mutations, were intercrossed with their respective parent lines and the F_1 then compared with both homozygous classes (JONES 1945). All hybrids were

significantly superior in one or more attributes. In a later article, JONES (1952) stated, ". . . but the results to date indicate that the differences involved are not single genes." No data have been published, although the above remark indicates that the assumption of a single gene difference was not correct.

MATERIALS AND METHODS

In long time inbred lines of corn occasional plants may be found which differ sufficiently from their full sibs in one or more morphological characteristics as to be easily recognizable. Several such off-type plants, thought to be the result of single gene mutations, have been found and maintained (table 1). A total of 14 pairs of lines, namely the original and its corre-

TABLE 1

Designation, symbol, nature, origin and history of mutants, and year of test.

Name of mutant	Genetic symbol	Nature of mutation	Found by or obtained from	Generations of selfing or sibbing		Test grown in
				Prior to mutation	After mutation	
Narrow leaf	*nl*	Recessive	Sprague	10	4	1952
Sugary	*su 205*	Recessive	Sprague	20	5	1952
Male sterile	*ms 317*	Recessive	Sprague	20	5	1952
Brachytic	*br*	Recessive	Sprague	20	2	1952
Green stripe	*gs*	Recessive	Sprague	5	3	1952
Sugary	*su GG824*	Recessive	Sprague	10	5	1952
Dwarf	*d SW1*	Recessive	Pioneer	5	3	1952
Dwarf	*d 187-2*	Recessive	Pioneer	5	3	1952
Crinkled	*cr*	Recessive	Sprague	5	4	1953
Small seed	*sm M14*	Recessive	Sprague	10	3	1953
Grass like	*gr*	Recessive	Rubis	20	3	1953
Red pericarp	*P*	Dominant	Sprague	10	10	1953
Male sterile	*ms 38-11*	Recessive	Sprague	20	4	1953
Brown midrib	*bm*	Recessive	Sprague	4	1	1953
Small seed	*sm 1373*	Recessive	Sprague	5	1	1953
Dwarf	*d 187-2*	Recessive	Pioneer	5	3	1953

sponding mutant line, were grown in 1951 in adjacent single row plots to make crosses between the pair as well as to sib some plants within each row. The mutant line always served as a male parent. Desired amounts of seed of each of the three possible genotypes *AA, Aa* and *aa* were obtained for only eight of the 14 pairs of lines, namely narrow leaf, sugary 205, sugary GG824, brachytic, green stripe, male sterile 317, dwarf SW1 and dwarf 187-2. The three genotypes of the eight mutants were grown in 1952 in eight separate randomized block designs with from 7 to 16 replications. These tests will be called "mutant tests." A plot consisted of a single 10-plant row, with plants spaced approximately 13 inches apart in the row. The following measurements were taken on an individual plant basis: plant height in centimeters, total weight of shelled seed per plant in grams, total number of kernels per

plant, kernel row number and ear length in millimeters. Ear diameter in millimeters, leaf width in millimeters, and number of tassel branches were recorded only in the narrow leaf mutant test.

Seed for seven more mutant tests was produced in 1952 and in the greenhouse in 1952/1953. They were crinkled, small seed M14, grasslike, red pericarp, male sterile 38-11, brown midrib and small seed 1373. Randomized block designs with the same plot size were used, except that the *aa*-genotype was omitted, because from the analyses of the previous year it was learned that the homozygous recessives were of no particular interest in this type of analysis. The mutant test of dwarf 187-2 was repeated in 1953, except for the omission of the homozygous recessive sibs. Characters measured in 1953 were the same as in 1952, except for the substitution of 100-kernel weight for kernel number. The mutant tests for brown midrib and red pericarp were inconclusive because in each case one of the parents, although presumed to be homozygous, was found to be heterozygous.

In 1952 seed was collected from heterozygous plants which were either selfed or backcrossed to the homozygous recessive stock. Their progenies were grown in an attempt to recover the homozygous recessive mutant type. Selfs were obtained for the lines narrow leaf, sugary 205, sugary GG824, and green stripe, and backcrosses for brachytic and dwarf SW1. For these mutants the double recessive segregates were phenotypically separable and only on them were data recorded. Such recovered or extracted homozygous recessives could be compared with or tested against the original recessive stock. These experiments are called " recovery tests " and had only two entries, namely the progeny of the selfed or backcrossed ear and a mechanical mixture of the dominant parent line and the original recessive mutant. In both entries data were collected only on the homozygous recessive mutants. The purpose of the mixture was to provide the smallest degree of bias in border effects by simulating the segregation in the entries planted with selfed or backcrossed seed. Recovery tests were laid out as randomized blocks and handled in the same manner as the mutant tests.

Within each mutant and recovery test an analysis of variance was performed for each attribute measured. Only data from the homozygous dominant and the heterozygous plants were used in the mutant tests in 1952 because the homozygous recessive class was constantly and distinctly inferior. Furthermore, the most interesting comparison is represented by the *AA* class vs. the *Aa* class. All mutant tests in 1953 had only two entries, namely, the *AA* vs. the *Aa* line. To improve classification or separation in both the mutant and the recovery tests three attributes were combined to form a discriminant function. The technique was developed by FISHER (1936) and demonstrated by COX and MARTIN (1937) and others. An analysis of variance was calculated for each attribute separately and for the discriminant. A t-test of the discriminant also was calculated.

TABLE 2

Means of the three genotypes AA, Aa and aa for each of the attributes taken on eight mutants in 1952.

Mutant	Geno-type	Height in centimeters	Number of seeds	Total kernel weight in grams	Kernel row number	Ear length in millimeters	Leaf width in millimeters	Number of tassel branches	Ear diameter in millimeters
	AA	156.18	795.51	141.20	12.02	151.44	81.53	13.79	40.59
nl	*Aa*	155.78	736.36	133.55	11.90	148.75	81.30	12.72	39.90
	aa	155.40	319.30	52.00	10.30	130.50	44.40	7.60	33.70
	AA	217.73	230.53	63.98	14.94	147.02			
su 225	*Aa*	218.93	286.06	81.75	16.59	159.10			
	aa	212.00	154.00	49.00	13.00	143.00			
	AA	230.13	553.78	103.20	14.09	199.24			
ms 317	*Aa*	226.16	518.18	97.18	14.27	200.36			
	aa	....	....	....	....	....			
	AA	134.82	460.19	107.71	18.58	173.88			
br	*Aa*	138.39	465.19	109.45	18.37	166.24			
	aa	84.80	341.50	70.70	18.30	156.30			
	AA	152.88	329.44	91.63	16.58	164.59			
gs	*Aa*	148.67	426.20	120.68	17.26	185.77			
	aa	139.20	236.20	47.30	15.20	147.20			
	AA	170.60	96.46	18.56	14.24	96.84			
su GG824	*Aa*	172.36	92.93	20.18	14.48	109.18			
	aa	182.60	76.40	10.60	14.50	96.80			
	AA	188.58	701.65	168.23	15.43	214.06			
d SW1	*Aa*	182.04	917.34	237.43	15.31	232.73			
	aa	92.30	426.90	81.40	15.20	174.90			
	AA	179.05	530.98	116.27	13.70	190.79			
d 187-2	*Aa*	192.46	724.61	185.82	15.72	221.61			
	aa	83.70	138.30	30.30	10.60	107.00			

EXPERIMENTAL RESULTS

Mutant tests

The means of the three genotypes for each of the eight mutant tests grown in 1952 are summarized in table 2. The homozygous recessive class was the least vigorous in all but two of the 38 cases for which measurements on recessives were available. The recessives were not included in the analyses of variance (table 4). The means of the mutant tests grown in 1953 are shown in table 3. The F-values for tests grown in 1952 and 1953 are presented in table 4.

In the narrow leaf mutant, comparing the dominant vs. the heterozygous class, all differences were in favor of the double dominant genotype, but only for kernel row number was the difference significant at the 5 percent level.

TABLE 3

Means of the two genotypes AA and Aa for each of the characters measured on five mutants in 1953.

Mutant	Genotype	Height in centimeters	Kernel row number	Ear length in millimeters	100-kernel weight in grams	Total kernel weight in grams
cr	*AA*	196.15	15.76	191.36	25.78	86.26
	Aa	222.38	17.47	221.29	27.46	139.17
sm M14	*AA*	180.76	16.85	210.28	20.83	124.82
	Aa	178.12	16.36	216.38	21.58	128.14
gr	*AA*	201.55	15.81	175.15	31.62	125.35
	Aa	190.07	15.11	175.79	30.40	113.62
sm 1373	*AA*	172.25	13.37	188.53	26.93	126.18
	Aa	173.11	13.60	184.73	26.68	116.95
d 187-2	*AA*	184.38	13.61	194.78	24.49	68.64
	Aa	207.85	14.40	216.74	26.07	132.42

In the sugary 205 test the heterozygous allele combination was superior to the homozygous dominant genotype and all differences except for plant height were highly significant. Plant height was the only attribute for which significant differences were found in the male sterile 317 and the brachytic line. In the former the difference was in favor of the dominant class; in the latter the heterozygotes were superior. In the green stripe test all analyses gave a highly significant F-value and, with the exception of plant height, the heterozygotes were superior to the dominant parent. The only significant difference obtained in the sugary GG824 line was for ear length, the heterozygote having longer ears. In both dwarf mutants differences in all but two of the attributes measured were highly significant; the heterozygotes always exhibited the greater mean. The test for the dwarf 187-2 mutant, which was repeated in 1953, showed the same trends as in 1952. The heterozygotes for crinkled exceeded significantly the homozygotes in all characters. In the small seed M14 all but one of the differences were non-significant. The same held

TABLE 4

The variance ratios obtained in the analyses of variance of the heterozygous vs. the homozygous dominant genotype for all attributes measured on all mutants in 1952 or 1953.

Mutant	Height	Number of seeds	Total kernel weight	Kernel row number	Ear length	100-kernel weight	Leaf width	Number of tassel branches	Ear diameter
nl	.04	1.11	.42	5.00*	4.10	....	.13	4.39	3.38
su 225	.56	10.73**	18.81**	66.00**	43.22**				
ms 317	5.77*	.54	.68	3.09	.21				
br	12.14**	.25	.19	.62	.56				
gs	13.75**	34.27**	47.03**	6.41*	65.27**				
su GG824	.47	.31	.66	.86	93.83**				
d SW1	1.53	19.99**	29.86**	.73	21.10**				
d 187-2	75.51**	72.18**	371.31**	115.37**	253.30**				
cr	136.26**	....	25.01**	115.00**	41.41**	8.21*			
sm M14	4.53	....	.68	6.03*	2.31	.79			
gr	32.21**	....	3.28	2.80	.03	5.71*			
sm 1373	.26	....	12.10**	1.10	2.25	.18			
d 187-2	205.93**	....	144.67**	24.38**	94.42**	17.78**			

The homozygous recessive entry was omitted in all analyses.
*Exceeds the 5 percent level of significance.
**Exceeds the 1 percent level of significance.

for the second small seed line. The homozygous dominant plants in the grass-like mutant test exceeded the heterozygotes significantly in plant height and 100-kernel weight. In the test of the male sterile 38-11 no stand was obtained.

For every one of the 12 mutant tests a discriminant function consisting of three attributes was calculated. Then each function's discriminatory value was evaluated by an F-test and a t-test, as shown in table 5. The relative value of each of the three attributes is represented by the magnitude of the coefficient attached to each selected character. For both dwarf mutants the same three characters were chosen, namely, plant height, total kernel weight and ear length. In the dwarf SW1 experiment, plant height was not significant, but nevertheless it had the highest discrimination coefficient demonstrating that neither the F- nor t-test provided an entirely reliable criterion in selecting the attributes, because possible correlations among the attributes are disregarded. In case of the dwarf 187-2 test, which was repeated in 1953, the same three attributes were chosen as for the 1952 test. The correlation coefficients changed considerably from year to year. The rank of the discriminatory value of the three attributes was unaltered but the magnitudes of the individual coefficients were quite dissimilar in the two years.

In general in every mutant test a highly significant F-value was obtained for the discriminant, and in all cases the misclassification frequency of the discriminant was lowered as compared to any single attribute.

Recovery tests

An attempt was made to secure selfed or backcrossed seed only for the mutant tests grown in 1952. No seed for the male sterile 317 was attempted. The backcross seed of the dwarf 187-2 heterozygote was lost accidentally. Seeds homozygous for sugary 205 and sugary GG824 germinated so poorly that both recovery tests were abandoned. Viability of plants homozygous for brachytic was reduced to such an extent that the final stand was too poor to make an analysis worthwhile.

In both the plots with segregating progenies and with mixtures, plant counts were undertaken at pollination and at harvesting time. Expected ratios were in many cases quite distorted, which probably was due to differential viability of the different genotypes. This differential viability was not always consistent in the two entries of a test.

Means and F-values are summarized in table 6. In the recovery tests for narrow leaf, green stripe and dwarf SW1 a discriminant was obtained and an F- and t-test made (table 7). The three attributes selected for the combined analysis are the same as for the discriminant of the mutant test with the exception of the narrow leaf recovery test where ear length was substituted for leaf width. The difference between the two sources of recessives for all three mutations were highly significant when the discriminant was used as means of separation. Misclassification frequencies ranged from < 15 to < 30 percent.

TABLE 5

Linear discriminants obtained, their significance and frequency of misclassification resulting in all mutant tests.

Mutant	Discriminant[a]	F-value	Error of misclassification using									
			Z	H	N	W	R	L	S	Lw	T	E
nl	.011,975T + .024,544E + .066,501L	3.38*	.30					.40			.40	.40
su 225	.001,258W − .298,451R − .025,156L	57.79**	.005			.25	.10	.15				
ms 317	.018,542H + .000,206N − .175,340R	6.83**	.20	.35	.45		.45					
br	−.036,130H + .129,945R + .002,471L	13.08**	.15	.30			.45	.45				
gs	.015,417H + .012,000W − .014,969R	31.63**	.05	.30		.15	.35					
su GG824	−.228,145W + 1.120,761R − .729,303L	106.17**	.0005			.45	.45	.025				
d SW1	.005,159H − .003,927W − .002,025L	25.63**	.10	.45		.20		.25				
d 187-2	−.059,918H − .037,687W − .056,983L	386.84**	.0005	.01		.005		.01				
cr	−.045,860H − .563,908R − .011,671L	187.90**	.0005	.05			.05	.15				
sm M14	.013,618H + .091,928R − .004,106L	7.92**	.20	.40			.35	.40				
gr	.033,532R + .094,600S + .003,433W	5.85**	.25			.35	.35		.30			
sm 1373	−.048,807R − .003,240L + .013,087W	8.94**	.20			.30	.45	.40				
d 187-2	−.061,740H − .004,807W − .023,267L	169.24**	.0005	.025		.025		.10				

[a]Symbols represent Z discriminant function; H plant height; N kernel number; W total kernel weight; R kernel row number; L ear length; S 100-kernel weight; Lw leaf width; T number of tassel branches; E ear diameter

TABLE 6

Means and F-values for all characters measured on four mutants in the recovery tests.

Mutant	Source of recessive and F-value	Plant height in centimeters	Kernel row number	Ear length in millimeters	100-kernel weight in grams	Total kernel weight in grams	Leaf width in millimeters	Number of tassel branches
	Original	155.60	9.97	138.36	18.20	66.65	39.93	7.09
nl	Extracted	154.57	10.35	129.41	17.83	63.23	43.16	8.20
	F-value	.10	2.63	1.91	.71	.20	7.57*	7.48*
	Original	80.80	17.33	166.13	20.13	66.33		
br	Extracted	80.40	18.60	164.60	21.00	94.80		
	F-value[a]	....	....	....	....	....		
	Original	121.76	14.61	139.78	24.18	31.80		
gs	Extracted	133.02	15.62	130.54	21.74	45.47		
	F-value	14.40**	2.98	1.56	1.31	6.87*		
	Original	107.38	15.44	197.58	21.52	101.65		
d SW1	Extracted	101.48	15.55	186.39	20.59	87.37		
	F-value	4.47*	0.00	8.02**	2.36	3.51		

[a]Not enough recessives were obtained to make an Analysis of Variance.
*Exceeds 5 percent level of probability.
**Exceeds 1 percent level of probability.

TABLE 7

Linear discriminants obtained, their significance and frequency of misclassification resulting in all recovery tests.

Mutant	Discriminant[a]	F-value	Errors of misclassification using						
			Z	H	W	R	L	Lw	T
nl	.016,787Lw + .048,057T − .000,518L	10.77**	.20	....	....	....	.45	.35	.35
gs	−.019,744H − .004,947W − .006,410R	9.78**	.15	.25	.30	.40	....		
d SW1	.000,883H + .001,612L − .000,118W	5.63**	.30	.40	.45	....	.40		

[a]For symbols see table 5.

DISCUSSION

Mutant tests

For breeding purposes a practical solution to the heterosis problem would consist in determining the relative importance of dominance and overdominance. SPRAGUE and MILLER (1950) have devised a scheme which may permit the evaluation of the relative importance of the two kinds of gene action *en masse*. Limited data have been presented (SPRAGUE and MILLER 1953) and are in agreement with the dominance hypothesis. An answer to the relative importance, however, would not provide critical evidence to the basic issue and would not permit phrasing a basic law for the individual and universal case. One of the major difficulties of the past has been the fact that many experiments reported in the literature may be explained equally satisfactorily by either the dominance or the overdominance hypothesis. It is a common belief among breeders and geneticists that both types of gene action are involved.

The phenotypic effect of a gene is a result of some primary component, such as catalytic or enzymatic action. Only in rare instances, if ever, are we able to measure these primary components. Ordinarily, genes are characterized only by their end effects. The latter is the case in this study because here a number of attributes were recorded on genotypes presumably differing by one or two alleles. The concept of dominance in such cases frequently needs to be restated in terms of each partial effect separately rather than considered " en masse," because the homozygous allele pair may have positive and negative partial effects upon the phenotype although the primary effect may be either minus or plus. Upon observing the sum of secondary effects, a combination of the two alleles appears to operate in the sense of single locus overdominance. The same holds for cases of close linkage in the repulsion phase.

The above demonstrates the difficulties involved in what may be called a phenotypic approach, and it emphasizes the necessity and possibilities of biochemical methods. But for biochemical studies to be effective on single locus heterosis requires genetic stocks which differ in only one allele. Such material at the present time cannot be secured. Fairly large numbers of single gene mutations or point mutations in long time inbred lines of maize are the closest approximation available at the present time.

The concept of point mutation is a working hypothesis. No cytological or genetical tools are available to classify positively a heritable change as a point mutation. Gross changes in the chromosomal make-up are detectable by cytological examination, but small unexposable inversions, duplications, deficiencies and rare cross-overs may give mutation-like effects. These difficulties are especially important in heterosis studies where X-ray induced mutations are used. When X-ray treatments are involved it appears probable that changes without phenotypically visible effects are likely to have occurred.

Another method to obtain lines which differ by one allele consists in con-

tinuous back-crossing of a mutant to a homozygous recessive stock. The expected end result is a second homozygous stock identical to the recurrent parent in all but the mutant locus. A second way of obtaining such lines is to cross two lines, select in each generation a progeny plant heterozygous for the locus studied while the other loci approach homozygosity by continued selfing. After an adequate number of selfed generations, contrasting lines are selected. The difficulty inherent in the production of isogenic lines by backcrossing or selfing is the unlikeliness of replacement of genes located close to the locus concerned. Hence, resulting lines may be largely isogenic but may differ with respect to short chromosomal segments on which the mutants are located. It seems reasonable that the best approximation to ideal isogenic conditions are cases where mutations occurred in long time inbred lines, clones, identical twins, etc. Neighboring chromosomal regions probably would remain constant.

Maize is a rather favorable subject for this kind of study. It is genetically and cytologically more thoroughly investigated than any other plant species and it can be easily selfed and crossed. Drawbacks are that we depend upon natural mutations of a conspicuous form which occur with low frequencies. The mutant stock as well as parent lines must be grown from time to time to maintain viable seed. Each such selfed or sibbed generation exposes the gene complex to new mutations. The approximate number of selfed or sibbed generations for the mutants and their original stocks is shown in table 1. Complete homozygosity of even long-time inbred lines cannot be claimed, because of the possibility of relic heterozygosity (heterozygosity not eliminated by inbreeding). There is no reliable information on the importance of relic heterozygosity or newly occurring mutations in inbred lines of maize. In spite of all these obstacles the use of natural mutations in inbred lines of corn appears to be one of the more critical techniques available for the investigation of single locus heterosis.

It is recognized that the 12 mutations in this study with which tests were completed are neither a random nor an adequate sample of all loci. They are all recessive mutations which probably mutate with higher than average frequency and have above average phenotypic appearance. In spite of these limitations, this sample is the largest collection of such mutations in maize specifically tested for their heterotic effect in supposedly isogenic lines. JONES (1944, 1945) described six heritable changes in corn and conducted tests similar to the one reported in this paper. A number of irregularities in segregating progenies and instability of lines were mentioned in the 1945 articles and he later (1952) stated that the differences were not of a single gene nature.

If heterozygosity *per se* is advantageous, then all heterozygous loci should exhibit overdominance, which was true for the six mutants reported by Jones. A more realistic assumption would be that an overdominant type of gene action exists for only a limited number of loci. In that event one would expect to find heterotic and non-heterotic loci in a random sample. The 12 mutants in this study seem to bear out this assumption. The loci sugary 205,

green stripe, dwarf SW1, dwarf 187-2 and crinkled definitely appear to be examples of heterotic allele combinations in that all or almost all variables were significantly higher in the heterozygotes (tables 2, 3 and 4). The contrasting group of alleles was comprised of narrow leaf, male sterile 317, grass-like and small seed 1373. Here none of the differences was in favor of the heterozygotes and if significant differences existed, the dominant parent was the better. The third or intermediate group was indefinite. Differences were small, varied from one attribute to the other and were rarely significant. Cases of this type were brachytic, sugary GG824 and small seed M14.

The above classification of the 12 mutants is based on the analysis presented in table 4, without utilizing the discriminant function approach. Separation of the two genotypic classes for all mutants can be improved by the use of the discriminants as seen from the lowered errors of misclassification (table 5). The validity of the discriminant function approach may be questioned on grounds that the attributes in many cases, were selected according to their F-values and not at random. However, under the assumption of a single gene difference it must be assumed that the magnitude of the means of the contrasting genotypes was due to this one locus.

Recovery tests

As discussed in the foregoing section, relic heterozygosity and new mutations constitute a source of variability which could invalidate results obtained in the mutant tests. In none of the previous reports in the literature on single locus heterosis had an attempt been made to investigate the importance of these factors. JONES (1952) stated without presenting any data, that in the material reported in 1945 ". . . some other changes must have been associated with the variable condition." Usually the authors justified their assumption of a single gene difference by establishing a monohybrid ratio.

In view of the two conceivable sources of variability in supposedly isogenic lines, and the lack of critical evidence in the literature in support of a single locus difference, the recovery tests were thought to be essential. Unfortunately, for reasons mentioned in Materials and Methods, only three of these experiments were analyzable, namely narrow leaf, green stripe and the dwarf SW1 mutants. The two latter loci were previously classified as possible heterotic loci, while the narrow leaf alleles showed no such gene action. In spite of the small number of recovery tests the results were surprisingly uniform in that all experiments gave highly significant F-values for the variance ratio in the analyses of variance of the discriminant functions. In the case of the two heterotic mutants this would indicate that the assumption of a single locus difference was not correct and that other sources of variability were present in the supposedly isogenic line pairs. The separation of the two sources of recessives for the green stripe and dwarf SW1 mutants was effective by the same combination of attributes as was used the previous year in the mutant tests. The significant F-value for the narrow leaf discriminant of the recovery test merely points out that here also the two members of the

isogenic line must have differed by more than the narrow leaf locus. The source of this additional variability may be new mutations arising during the generations of selfing or sibbing following the discovery of the mutant or it may be relic heterozygosity present in the inbred line at the time the mutation occurred. In corn it would be rather difficult to eliminate these two possibilities, unless an intensive search is undertaken for mutations in one-year-old inbred lines derived by the haploid method. If the indication of unsuspected variance in isogenic lines, as disclosed by this study, are of general nature, then practically all of the most critical experiments of the past are rendered invalid.

The disproval of the assumption underlying the mutant tests does not exclude that these loci may have had overdominance-like effects. But it does point out that what other investigators have labeled as single gene heterosis may likely be due to a multigenic diversity. Further, no conclusions can be drawn in respect to the degree of importance of cumulative small heterotic effects nor that such effects may not be of importance. On the other hand, it is felt that present evidence on types of gene action and interaction such as dominance and epistasis can account for the heterosis phenomenon in a satisfactory manner.

SUMMARY

1. Dominance and/or overdominance are currently and commonly the two hypotheses advanced for explaining the heterosis phenomenon. Superiority of the heterozygous genotype over both homozygous classes for a single locus with a homozygous genetic background would be evidence for one type of overdominance.

2. Seed of the three possible genotypes *AA, Aa* and *aa* for each of 15 mutants, which had occurred in long time inbred lines, was produced and tested in randomized block experiments. The tests in 1952 included all three genotypes, while in 1953 the homozygous recessive class was omitted. Measurements on an individual plant basis were recorded and a total of 12 mutants analyzed.

3. Selfing or backcrossing plants heterozygous for the mutation resulted in recovered homozygous recessives which were compared in randomized blocks with the original recessives. It was possible to analyze recovery tests for only three mutants (narrow leaf, green stripe and dwarf SW1).

4. A separate analysis of variance was calculated for each attribute measured in each of the mutant and recovery tests. For each mutant and recovery test three attributes were chosen and a discriminant function calculated. Analyses of variance for the discriminant were presented. T-tests of the discriminant and the individual attributes permitted a comparison of misclassification frequencies.

5. According to the results obtained in the mutant tests, the 12 mutants were classified into three groups: 1) mutants where the heterozygous genotype was distinctly superior in all or many of the attributes measured, 2) mutants

where no heterotic behavior was apparent, 3) mutants where a classification was intermediate or erratic.

6. The variance ratio of the discriminant was highly significant in all three recovery tests indicating that the two sources of plants homozygous for the recessive mutant were not identical with respect to loci other than the mutant genes. These results preclude the acceptance of heterotic single loci effects as obtained in the mutant tests. Similarly, previously published experiments by a number of authors might have revealed such discrepancies if recovery tests had been conducted.

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