NATURAL MUTATIONS IN INBRED LINES OF MAIZE AND THEIR HETEROTIC EFFECT. II. COMPARISON OF MOTHER LINE VS. MUTANT WHEN OUTCROSSED TO UNRELATED INBREDS¹

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THE utilization of heterosis in corn was first outlined by SHULL (1909), and the procedures put forth by him are essentially those in practice today. For the past three decades plant breeders have conducted empirical investigations aimed at a more efficient exploitation of this great genetic phenomenon. Improved methods found have resulted in some advancement. The practical aspects of heterosis would be simplified for the breeder if the relative importance of dominance and overdominance could be evaluated, but geneticists interested in gene action would still need additional information.

The experiments described in Part I of this series of papers (SCHULER 1954) involved a comparison of the homozygous (AA) genotype versus the heterozygous (Aa) genotype on a highly inbred and therefore highly homozygous genetic background. It was demonstrated that the apparent heterotic effects of single loci for some mutants actually may have been due to an unknown number of concealed additional heterozygous loci. The results did not exclude the possibility of overdominance but merely questioned the basic assumption of a single allelic difference. It was pointed out previously that experiments reported in the literature where overdominance was indicated also may have been based on incorrect assumptions regarding single gene differences, since few attempts were made to investigate concealed genetic variability.

It was felt that the same comparison, i.e. AA vs Aa ought to be extended to, and repeated in, a genetically heterozygous medium by outcrossing both members of a line pair to inbred lines that were not related to motherline and mutant. Single locus overdominance, if existent, may either be of no consequence, or may be potent to a detectable degree, in a system of heterozygous loci.

SINGLETON (1943) in a short note described a semi-dwarf mutant that occurred in an inbred line of sweet corn. When the mutant and the original inbred line were outcrossed to a common inbred parent, the cross involving the mutant in some cases yielded significantly more than the outcross with the original line carrying the dominant factor for the locus under consideration. No data were presented. In a subsequent note (1947) he reported that when inbred lines were extracted from the above mentioned cross, motherline $\times$ mutant, variations such as germless seed, brittle

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and virescent were observed. He mentioned that mutations for combining ability had occurred also. No data were given.

SPRAGUE and MILLER (1953) presented preliminary evidence in favor of the relative importance of a dominance type of gene action in corn. Their evidence needs to be substantiated by results from additional cycles of selection. No control of genetic background in the form of homozygosity or homogeneity was feasible because of the nature of the experiment. Extensive experimentation designed to estimate the ratio of dominance to additive genetic variance has been reported in open pollinated varieties of corn as well as in populations derived from crosses of inbred lines by ROBINSON *et al.* (1949 and 1955). No evidence for overdominance in plant and ear height, ear number, ear length and ear diameter was obtained. For yield there was no clear indication of overdominance in the cases involving open pollinated varieties, while F_1 populations derived from intercrossing inbreds showed overdominance or possibly pseudo-overdominance due to linkages. These experiments also did not involve control of all or even most of the genotype but they yield information on average gene action relative to the particular population under study. A total of 52 chromosomes from natural populations of *Drosophila willistoni* were tested for viability of heterozygous lethals by CORDEIRO (1952). Five of these chromosomes were selected by CORDEIRO and DOBZHANSKY (1954) for their apparent superior viabilities of the lethal heterozygotes, which was contrary to the effect of the average of the 52 chromosomes. Outcrosses to a number of stocks and comparing the normal/normal versus the normal/lethal revealed a slightly beneficial effect of the lethal bearing chromosomes. DOBZHANSKY and SPASSKY (1947) made a second or fourth chromosome homozygous in seven lines. Each line was divided into four sublines, two of which were kept homozygous with respect to the second or fourth chromosome and the other two had a "balancing" chromosome introduced into them which served as a crossover suppressor. Furthermore one of each of the two groups received X-ray treatment. The lines were then propagated over 50 generations. The results indicated partial restoration of the vigor and fertility that had been decreased upon making the chromosome homozygous. Their techniques permitted detection of deterioration or improvements in the viability of homozygotes for the chromosomes studied, but not of heterozygotes.

MATERIAL AND METHODS

A total of eight pairs of inbred lines were available. Each pair consisted of an original inbred line or motherline and the identical line except for carrying a homozygous recessive mutant. A brief description of these lines was presented in table 1 of part I (SCHULER 1954) of this study. Each line carried a different mutation. These were as follows: crinkled leaf, narrow leaf, brachytic stalk, green stripe, two dwarf lines and two small seed lines. The two dwarf and small seed mutants were not allelic. The three inbred lines serving as testers were derived by selfing naturally occurring monoploids (CHASE 1952) developed by DR. S. S. CHASE. The first line (D_I) went through two generations of selfing after diploidization of the monoploid. The second line ($D_{I'}$) was identical to the first except for an additional generation of selfing as a diploid. The third tester (D_{II}) was unrelated to the first two, stemming from another genetic source. It had passed through two diploid generations. The three tester

inbreds are symbolized by $D_I D_I$, $D_{I'} D_{I'}$ and $D_{II} D_{II}$, where the capital D stands for the respective dominant allele and the Roman subscript for the tester source. Similarly the mutant line pairs may be designated as $D_M D_M$ and $d_M d_M$, where D and d stand for the gene under study and the subscript M indicates that the allele came from the line pair.

Each of the eight line pairs was grown in 1953 in a single row and used as male parent in an outcross to the unrelated tester inbreds. No attempt was made to resort to the same male plant in each row as pollen sources for all outcrosses, nor was a bulked pollen sample taken consistently. The first six of the above enumerated mutants and their motherlines were outcrossed to all three inbred testers. Due to different maturities the two pairs involving the small seed mutation could not be crossed to tester $D_{II} D_{II}$. These crosses resulted in six F_1 populations ($D_M D_I$, $D_M D_{I'}$, $D_M D_{II}$, $d_M D_I$, $d_M D_{I'}$, $d_M D_{II}$) for the first six mutants, and four others ($D_M D_I$, $D_M D_{I'}$, $d_M D_I$, $d_M D_{I'}$) for the two small seed mutants.

The randomized complete block design adopted was such that each line pair was treated as a separate experiment allowing statistical treatment of intra-line pair comparisons only. The six and four entries respectively of each experiment, were grown in ten replications in 1954 near Ames, Iowa. Each plot consisted of a single row with ten plants spaced approximately 13 inches apart. Four individual plants, competitive plants whenever possible, were taken at random and the following measurements recorded: plant height in centimeters, kernel row number, ear length in millimeters, ear diameter in millimeters, weight of 100 seeds in grams, weight of shelled grain in grams and weight of unshelled ears in grams. Lodging prevented the collection of meaningful data on plant height in the narrow leaf experiment.

For each attribute within each experiment an analysis of variance of the following pattern was calculated:

Source	Analysis of variance <i>d.f.</i>	<i>E(M.S)</i>
Total	$srt - 1$	
Replications	$r - 1$	
Treatments (crosses)	$t - 1$	$\sigma_e^2 + s \sigma_{rt}^2 + rs \sigma_t^2$
Repl. $\times$ treatments	$(r - 1)(t - 1)$	$\sigma_e^2 + s \sigma_{rt}^2$
Sampling	$(s - 1)rt$	σ_e^2

where r represents the number of replications, t of treatments and s of samples (plants within plots). The treatment sums of squares were divided into $t - 1$ orthogonal comparisons:

Symbol	Description
C_1	Homoz. dominants vs. heteroz. = $(D_M D_I + D_M D_{I'} + D_M D_{II}) - (d_M D_I + d_M D_{I'} + d_M D_{II})$
C_2	Tester D_I vs tester $D_{I'}$ = $(D_M D_I + d_M D_I) - (D_M D_{I'} + d_M D_{I'})$
C_3	Tester $D_I + D_{I'}$ vs tester D_{II} = $(D_M D_I + d_M D_I + D_M D_{I'} + d_M D_{I'}) - (2D_M D_{II} + 2d_M D_{II})$
I_1	Interaction $C_1 \times C_2$ = $(D_M D_{I'} - D_M D_I) + (d_M D_I - d_M D_{I'})$
I_2	Interaction $C_1 \times C_3$ = $(2D_M D_{II} - D_M D_I + D_M D_{I'}) + (d_M D_I + d_M D_{I'} - 2d_M D_{II})$

The comparisons C_3 and I_2 were not available in the two experiments which had 4 treatments. For testing the variance ratios for treatments and individual comparisons the replication $\times$ treatment interaction mean square was used.

Each sampling sum of squares was partitioned into as many parts as there were treatments to obtain information on within treatment variances and ratios of such mean squares.

EXPERIMENTAL RESULTS

The means of the six (four) F_1 populations for each of the eight mutants obtained from the eight experiments are presented in table 1. The variance ratios testing the differences among these means are presented in table 2. Differences generally were significant with the exception of the small seed 1373 line pair. Upon examination of table 1 it becomes obvious that the high F ratios in most cases were due largely to the unlike effect of the tester parent I and I' versus II. This result was anticipated. Table 3 contains the variance ratios for the orthogonal comparisons. Under each F value the linear combination obtained by adding and subtracting the treatment totals according to the orthogonal linear forms is placed in parentheses. Table 3 reveals the main points of the experiments.

The results can be viewed in terms of individual line pairs, of attributes, or of orthogonal comparisons over all line pairs. A brief description of the results obtained for each line pair may suffice.

The experiment involving the crinkled lines gave significant variance ratios for all attributes (table 2). This also was the case for contrasts C_3 of table 3, indicating a differential combining ability among the two tester sources. The direction of these differences varied from one trait to the next. The comparison C_1 , which was considered the most important aspect of the experiments, indicated that the recessive crinkled allele when combined with the dominant was superior to the homozygous dominant genotype for plant height, kernel row number and ear length but inferior for ear diameter. Yields did not differ significantly. The comparison C_2 was chosen to reveal and test differences between tester I and I'. In no case were variance ratios high. Only one of the interactions gave a significant F ratio.

The experiments with the narrow leaf and green stripe gave no discrimination between the $D_M D_T$ and $d_M D_T$ genotypes (T stands for Tester). Significance was obtained between the two tester sources (C_3). Of the few significant F values in the brachytic line experiment, kernel row number was significant in comparison C_1 , C_3 and I_2 . The results of the two dwarf mutants were similar in that in most attributes the differences between testers were significant (C_3) and in some instances also the comparison involving homozygous dominants versus the heterozygotes. The only cases in all of the experiments in which $D_M D_T$ vs $d_M D_T$ differed in yield were in the dwarf SW1, dwarf 187-2 and the small seed M14 mutant. However, the homozygous dominant genotype exceeded the heterozygous genotype for both dwarf mutants and the small seed M14 mutant. The two testers I and I' differed significantly in the small seed M14 test for plant height, ear diameter and both yield measurements. Furthermore, some interactions were significant.

The mean squares on a per plant basis for sampling variances are too bulky for

TABLE 1

Means for each measured attribute in four or six crosses with each of eight mutants

Mutant	Genotype	Plant height	Kernel row number	Ear length	Ear diameter	100 kernel weight	Weight of shelled grain	Weight of unshelled grain
<i>cr</i>	<i>d_MD_I</i>	228.27	18.35	242.92	46.10	34.75	233.35	272.20
	<i>d_MD_{I'}</i>	225.15	17.70	246.15	45.90	34.62	231.25	271.50
	<i>d_MD_{II}</i>	238.67	20.55	227.15	49.30	32.87	259.65	296.95
	<i>D_MD_I</i>	217.03	17.05	232.25	47.35	35.50	245.75	285.37
	<i>D_MD_{I'}</i>	215.10	16.70	233.75	47.00	35.47	228.80	266.12
	<i>D_MD_{II}</i>	235.77	19.80	222.02	51.12	33.60	268.10	303.85
<i>nl</i>	<i>d_MD_I</i>		14.00	200.15	43.62	34.05	187.47	219.17
	<i>d_MD_{I'}</i>		14.35	198.70	43.67	31.50	180.75	208.70
	<i>d_MD_{II}</i>		17.40	200.47	47.20	31.15	211.60	240.80
	<i>D_MD_I</i>		14.65	201.27	44.12	33.32	185.30	215.90
	<i>D_MD_{I'}</i>		14.45	198.00	43.87	32.55	172.05	201.80
	<i>D_MD_{II}</i>		17.50	197.55	46.52	31.07	193.20	222.27
<i>br</i>	<i>d_MD_I</i>	182.67	18.55	215.25	48.40	30.67	214.80	254.97
	<i>d_MD_{I'}</i>	185.40	18.40	207.65	47.70	28.97	196.75	238.50
	<i>d_MD_{II}</i>	203.77	19.05	212.67	47.67	28.65	209.42	246.52
	<i>D_MD_I</i>	188.25	17.90	210.57	47.60	30.47	205.52	248.62
	<i>D_MD_{I'}</i>	189.40	18.20	206.02	47.62	29.60	201.00	243.90
	<i>D_MD_{II}</i>	202.77	21.55	213.65	48.97	28.10	205.35	249.05
<i>gs</i>	<i>d_MD_I</i>	200.25	16.75	230.30	48.30	27.30	191.85	233.35
	<i>d_MD_{I'}</i>	202.37	17.10	214.97	48.77	28.72	207.00	251.25
	<i>d_MD_{II}</i>	215.45	22.15	207.90	51.32	27.05	236.57	273.62
	<i>D_MD_I</i>	208.00	17.90	220.85	48.52	25.45	196.37	238.62
	<i>D_MD_{I'}</i>	204.85	17.00	228.02	48.07	27.42	215.62	256.60
	<i>D_MD_{II}</i>	211.70	20.35	204.62	49.92	27.10	232.65	272.90
<i>d</i> SW1	<i>d_MD_I</i>	181.05	17.10	215.30	47.17	29.95	219.00	253.82
	<i>d_MD_{I'}</i>	175.75	16.85	221.80	47.15	29.65	205.52	238.57
	<i>d_MD_{II}</i>	219.77	19.20	227.57	49.02	30.70	247.07	283.77
	<i>D_MD_I</i>	217.72	16.15	225.62	48.22	31.85	226.55	270.95
	<i>D_MD_{I'}</i>	219.57	15.65	228.75	48.27	32.92	216.90	263.22
	<i>D_MD_{II}</i>	236.32	18.45	218.92	50.17	30.87	259.25	302.25
<i>d</i> 187-2	<i>d_MD_I</i>	226.07	16.45	235.37	46.40	29.47	232.52	269.02
	<i>d_MD_{I'}</i>	226.42	17.00	238.97	47.92	30.67	246.50	287.72
	<i>d_MD_{II}</i>	237.20	21.20	221.42	49.70	27.47	248.15	278.00
	<i>D_MD_I</i>	218.85	15.70	239.25	47.32	32.02	256.55	293.55
	<i>D_MD_{I'}</i>	214.57	16.00	238.52	47.50	30.95	244.50	282.80
	<i>D_MD_{II}</i>	239.27	19.95	231.57	49.67	38.67	267.65	298.92
<i>sm</i> M14	<i>d_MD_I</i>	193.05	15.75	193.07	43.90	29.60	160.82	189.25
	<i>d_MD_{I'}</i>	206.10	16.80	233.00	46.37	30.55	216.77	253.82
	<i>D_MD_I</i>	206.92	16.70	232.97	46.42	30.20	214.87	250.65
	<i>D_MD_{I'}</i>	205.37	16.50	223.00	46.52	30.32	215.67	252.75
<i>sm</i> 1373	<i>d_MD_I</i>	222.82	15.30	221.20	44.40	32.07	206.82	242.55
	<i>d_MD_{I'}</i>	221.32	15.50	220.55	45.37	32.05	220.30	258.67
	<i>D_MD_I</i>	220.15	15.75	220.62	45.12	32.20	215.15	251.35
	<i>D_MD_{I'}</i>	219.35	15.00	213.32	44.82	31.87	191.05	225.42

TABLE 2

Variance ratios from the analyses of variance of the attributes measured in eight mutants

Mutant	Plant height	Kernel row number	Ear length	Ear diameter	100 kernel weight	Weight of shelled grain	Weight of unshelled grain
<i>cr</i>	12.88**	40.78**	7.08**	21.85**	2.90*	4.05**	2.67*
<i>nl</i>		40.47**	.24	14.12**	3.94**	3.69**	2.62*
<i>br</i>	11.46**	42.63**	1.60	1.72	2.01	.66	.39
<i>gs</i>	6.51**	6.56**	1.93	4.01**	2.12	5.58**	3.42*
<i>d</i> SW1	62.51**	21.90**	1.09	8.97**	5.31**	7.73**	6.83**
<i>d</i> 187-2	9.19**	129.63**	3.27*	12.91**	6.49**	2.34	1.65
<i>sm</i> M14	9.74**	3.59*	6.34**	4.82**	1.20	6.00**	5.95**
<i>sm</i> 1373	.40	.73	1.45	1.72	.05	2.97	2.75

* Significant at the 5% level.

** Significant at the 1% level.

inclusion (a copy is on file in the Genetics Editorial Office). The values indicate that sampling variances in general were homogenous. No statistical test for homogeneity of variance was made since no simple and satisfactory method was available and BARTLETT'S test for homogeneity was known to be sensitive for non-normality. Variance ratios of the form $D_M D_{I'}/D_M D_I$ and $d_M D_{I'}/d_M D_I$ may shed some light on the problem of the observed increase of the plant to plant variability in successive generations of diploidized monoplids. These variance ratios were one-tail tests with $F = 1.84$ and 2.38 at the 5 percent and 1 percent level of probability respectively. All ratio tests were based on 30 and 30 degrees of freedom and are presented in table 4.

The test involving the brachytic and small seed M14, and to a lesser extent the dwarf 187-2 and green stripe line pairs gave some significant deviations indicating a difference between the two tester lines I and I'. The variance ratios intended to reveal differences in the genetic composition of mutants and motherline seemed to have no consistent trend. An accumulation of significant values in the crinkled test for tester II and the small seed M14 test for tester I was noted.

DISCUSSION

In part I of this series the original inbred line was crossed with its counterpart which was thought to be genetically identical except for a mutant allele. Genetic identity, which requires complete homozygosity could not be met, however. Heterozygosity persisted even after many generations of inbreeding. Explanations as to the source of such remnant heterozygosity have been put forth, but which of the possible forces are important remains to be demonstrated. Very likely mutations may create variability but other factors such as selective bias in favor of more heterozygous individuals, the pure chance in inbreeding coupled with the number of loci involved, the mechanical procedures used by breeders or as yet unrecognized phenomena may account for such variability. Remnant heterozygosity in connection with the control of the genetic medium may have a substantial effect if both homozygosity and homogeneity of genotypes are the critical assumptions. However, it

TABLE 3

The magnitude of the differences and the variance ratios for all attributes measured in three, or four orthogonal comparisons among the four or six crosses with eight mutants

Mutant	Comparison	Plant height	Kernel row number	Ear length	Ear diameter	100 kernel weight	Weight of shelled grain	Weight of unshelled grain
<i>cr</i>	C ₁	13.70** (-968)	26.85** (-122)	11.15** (-1128)	15.37** (167)	2.39 (93)	.86 (736)	.41 (588)
	C ₂	.89 (202)	4.33* (40)	.47 (-189)	.40 (22)	.02 (6)	1.38 (762)	1.13 (798)
	C ₃	46.92** (-2534)	171.44** (-436)	22.56** (2269)	92.70** (-580)	12.08** (296)	17.11** (-4654)	10.78** (-4256)
	I ₁	.05 (48)	.39 (12)	.06 (-69)	.03 (-6)	.01 (4)	.84 (-594)	.98 (-742)
	I ₂	2.81 (620)	.92 (32)	1.15 (513)	.74 (52)	.01 (-6)	.06 (278)	.03 (240)
<i>nl</i>	C ₁		1.88 (34)	.11 (-180)	.00 (1)	.03 (10)	2.94 (-1171)	2.01 (-1148)
	C ₂		.09 (-6)	.61 (189)	.00 (8)	7.14* (133)	2.05 (799)	2.21 (983)
	C ₃		198.60** (-494)	.04 (83)	68.53** (-486)	10.47** (279)	12.10** (-3361)	7.93* (-3223)
	I ₁		1.18 (-22)	.09 (-73)	.13 (-12)	2.03 (71)	.22 (-261)	.05 (-145)
	I ₂		.39 (-22)	.36 (-251)	1.95 (-82)	.05 (-19)	1.15 (-1037)	.88 (-1075)
<i>br</i>	C ₁	1.72 (343)	10.87** (66)	.59 (-213)	.16 (17)	.00 (-5)	.23 (-364)	.01 (63)
	C ₂	.53 (-155)	.96 (-16)	4.61* (486)	.61 (27)	3.18 (103)	2.13 (903)	1.47 (848)
	C ₃	53.38** (-2695)	132.58** (-326)	1.80 (-526)	1.75 (-79)	6.19* (249)	.18 (-459)	.03 (-206)
	I ₁	.08 (-63)	1.21 (18)	.29 (122)	.71 (29)	.33 (33)	.76 (541)	.45 (470)
	I ₂	1.58 (-463)	68.31** (234)	.71 (330)	5.43* (139)	.37 (-61)	.01 (125)	.04 (240)
<i>gs</i>	C ₁	1.37 (259)	.13 (-30)	.00 (13)	1.52 (-75)	3.10 (-124)	.23 (369)	.20 (369)
	C ₂	.05 (41)	.10 (22)	.30 (326)	.00 (-1)	5.60* (-136)	4.83* (-1376)	3.89 (-1435)
	C ₃	24.62** (-1553)	29.63** (-650)	7.02* (2764)	16.78** (-353)	.05 (24)	22.17** (-5104)	12.90** (-4529)
	I ₁	1.36 (-211)	.53 (-50)	2.23 (900)	.55 (-37)	.15 (22)	.07 (164)	.00 (3)
	I ₂	5.13* (-709)	2.43 (-186)	.16 (-406)	1.16 (-93)	1.71 (130)	.60 (-840)	.20 (-483)
<i>d</i> SW1	C ₁	167.15** (3882)	16.69** (-116)	.43 (-276)	12.47** (133)	17.01** (214)	3.05 (1244)	8.21** (2410)

TABLE 3—continued

Mutant	Comparison	Plant height	Kernel row number	Ear length	Ear diameter	100-kernel weight	Weight of shelled grain	Weight of unshelled grain
	C ₂	.31 (138)	1.67 (30)	.48 (236)	.00 (-1)	.54 (31)	2.53 (925)	1.79 (919)
	C ₃	123.76** (-4724)	90.48** (-382)	.89 (560)	32.36** (-303)	.45 (49)	32.96** (-5787)	23.94** (-5819)
	I ₁	1.36 (286)	.18 (-10)	2.02 (486)	.01 (3)	1.69 (55)	.07 (153)	.19 (301)
	I ₂	19.93** (-1896)	.42 (26)	1.65 (-762)	.00 (5)	6.92* (-193)	.05 (217)	.03 (-193)
<i>d</i> 187-2	C ₁	4.64* (-680)	36.42** (-120)	2.11 (543)	.27 (19)	6.36* (161)	5.01* (1701)	3.86 (1621)
	C ₂	.37 (157)	4.46* (-34)	.14 (-115)	5.23* (-68)	.01 (-5)	.00 (-37)	.22 (-318)
	C ₃	36.09** (-2681)	605.19** (-686)	12.18** (1845)	55.55** (-384)	23.00** (433)	3.54 (-2021)	.50 (-830)
	I ₁	.51 (-185)	.38 (-10)	.32 (-173)	3.29 (-54)	3.04 (-91)	3.04 (-1081)	3.06 (-1178)
	I ₂	4.33* (929)	1.16 (-30)	1.63 (675)	.18 (-22)	.03 (-17)	.35 (639)	.58 (890)
<i>sm</i> M14	C ₁	9.75** (526)	1.11 (26)	3.97 (1196)	5.34* (107)	.20 (15)	5.56* (2118)	5.42* (2413)
	C ₂	7.46* (-460)	1.90 (-34)	3.98 (-1198)	4.95* (-103)	2.08 (-43)	6.38* (-2270)	6.62* (-2667)
	I ₁	12.02** (-584)	4.10 (-50)	11.06** (-1996)	4.21* (-95)	1.23 (-33)	6.04* (-2206)	5.81* (-2499)
<i>sm</i> 1373	C ₁	.75 (-186)	.00 (-21)	.36 (-312)	.08 (7)	.00 (-2)	1.99 (-837)	1.99 (-978)
	C ₂	.23 (2)	1.03 (22)	1.63 (318)	1.12 (-27)	.11 (14)	.51 (425)	.32 (392)
	I ₁	.02 (28)	3.08 (-38)	1.14 (-266)	4.00 (-51)	.08 (-12)	6.40* (-1503)	5.87* (-1682)

* Significant at the 5% level.

** Significant at the 1% level.

might carry less weight if the contrast *DD* vs *Dd* is made on a highly heterozygous genetic background as was accomplished in this experiment by taking unrelated inbreds as tester parents.

The *Drosophila* experiment of CORDEIRO (1952) and others, where normal/normal was compared with the normal/lethal, deviated from this approach in that inferences about viability effects of a heterozygous lethal depended on the assumption of random genetic background. Results obtained with *Drosophila* may be further complicated by linkage effects. The latter limitation seems especially to impair conclusions about single locus heterosis in *Drosophila*. Even though some of these handicaps can be overcome with maize, it is unlikely that one might, beyond doubt,

TABLE 4

Ratios of within-cross variance for eight line pairs and seven attributes

Mutant	Variance ratio	Plant height	Kernel row number	Ear length	Ear diameter	100 kernel weight	Weight of shelled grain	Weight of unshelled grain
<i>cr</i>	$D_M D_I' / D_M D_I$	1.38	1.05	1.07	.66	1.05	1.00	1.10
	$d_M D_I' / d_M D_I$	.80	.64	1.14	.94	.75	1.44	1.38
<i>nl</i>	$D_M D_I' / D_M D_I$		1.17	1.80	.98	.69	.72	.70
	$d_M D_I' / d_M D_I$		1.50	2.77**	1.24	3.85**	.61	.60
<i>br</i>	$D_M D_I' / D_M D_I$	.65	1.08	3.62**	2.62**	1.70	4.40**	4.53**
	$d_M D_I' / d_M D_I$	.67	.90	3.24**	5.03**	4.82**	3.23**	4.81**
<i>gs</i>	$D_M D_I' / D_M D_I$	1.48	.80	1.07		.45	.61	.71
	$d_M D_I' / d_M D_I$	2.60**	1.50		1.60	1.43	2.28**	2.51**
<i>d</i> SW1	$D_M D_I' / D_M D_I$	1.05	2.02*	.69	1.04	.91	1.03	.92
	$d_M D_I' / d_M D_I$	1.39	.67	1.58	1.30	.59	2.21	2.05*
<i>d</i> 187-2	$D_M D_I' / D_M D_I$	.95	.60	1.36	1.11	1.44	.68	.74
	$d_M D_I' / d_M D_I$	.93	.80	.68	2.23*	.59	2.31*	2.31*
<i>sm</i> M14	$D_M D_I' / D_M D_I$	.60	1.58	4.12**	4.80**	1.38	2.87**	3.05**
	$d_M D_I' / d_M D_I$	.24	.34	.36	.50	1.00	.57	.53
<i>sm</i> 1373	$D_M D_I' / D_M D_I$	1.40	.89	.83	.93	1.67	.77	.58
	$d_M D_I' / d_M D_I$	.84	1.39	.67	.40	.95	1.12	1.15

* Significant at the 5% level.

** Significant at the 1% level.

secure two isogenic lines that differ by one allele only. Barring pseudo-overdominance effects of linkage, the need for complete isogenicity may be evaded. The following proposal for future work is advanced. Two highly isogenic lines differing by one conspicuous mutation are crossed and advanced to the F_2 or F_n generation to allow recombinations. This would tend to smooth out any effects of additional heterozygous loci and their interactions. The two genotypes DD and Dd would have an equal chance to combine with either allele of any other heterozygous locus. Such studies already are under way.

The data obtained on shelled and unshelled grain as a measure of yield did not support the hypothesis of overdominance. No significant F value obtained for yield favored the Dd genotype. These results were consistent for all mutants regardless of their behavior when tested on a highly homozygous background. If overdominance existed it was of such small effect in terms of the total heterosis that it could not be measured. These results are contrary to those obtained for the one mutation reported by SINGLETON (1943). However, in a later note (1947) he mentioned that mutations for combining ability also had occurred.

It is conceivable that many moderate overdominance effects on a number of loci, if cumulative, could be of determinable magnitude and therefore overdominance, unimportant on a single locus, may be of consequence because of its additive effects between loci. If this were the case one would expect results contrary to those reported by ROBINSON *et al.* (1949, 1955) and SPRAGUE and MILLER (1953).

No other attribute gave as consistent a picture as yield. For example in kernel row number the Dd genotype exceeded the DD class in the crinkled, dwarf SW1 and

dwarf 187-2 experiments, while the reverse was true for the brachytic test. Ear diameter was significant in the *DD* plants in two tests, in the *Dd* plants in one, and non-significant in the remainder.

In terms of individual experiments, the tests involving crinkled had three attributes significantly superior in the *Dd* class and in one the *DD* class was the better. In the small seed M14 test the homozygotes consistently exceeded the heterozygotes and the *F* value for plant height, ear diameter and yields had a probability of less than 5 percent. Small differences for all attributes were found for the green stripe, narrow leaf and small seed 1373 tests. The former (green stripe) was classified in the previous paper as a possible overdominant locus, while for the latter two no such effects were postulated. Those results seem to support the impression that with respect to this sample of loci, single locus heterosis effects if present were of no weight in a heterozygous genetic medium. Possible exceptions such as crinkled may need further study, although for yield the conclusions hold here as well.

The problem of heterozygosity remaining after many generations of selfing is widely recognized among plant breeders. It is tied directly into the assumptions employed in the preceding study and to a lesser extent in the experiment reported here. Inbreds derived by diploidizing monoploid plants are phenotypically extremely uniform but in succeeding generations this uniformity seems to deteriorate to such a degree that phenotypic plant to plant variance is very noticeable. Such an increase in variability within double monoploid inbred lines, if real, can not be explained by relic heterozygosity but must have originated in the homozygous gene complex by mutational changes. This observation by the writers and other workers in the field has not been evaluated in experimental trials. If subsequent data substantiate the observation then our present opinion on mutation rates, mutation effects, etc. may need to be revised before extending it to multigenic metric characters. Tests are now under way to determine whether an increase in variability occurs to a measurable degree.

Because little concrete information is available it was felt that by taking individual plant data, the within-cross variances and their ratios so obtained, while not the primary objective of the experiment, might yield some supplementary information. The results are summarized in table 4. Deficient and preliminary as they may be, they indicate nevertheless that plant to plant variance and variance between motherline and mutant could be critical factors. Furthermore, occasional *F* ratios of comparison *C*₂ in table 3 reached significant levels. However, it would be rather surprising if one additional sexual cycle gave rise to enough mutations for combining ability so as to result in a constantly altered performance of the line. Investigations aimed at an explanation of the genetic causes of variability within inbred lines are needed for the breeder of naturally and artificially self-pollinated crops as well as for future research. A logical extension of such analyses would lead to an examination of the magnitude of variances among homozygous and/or homogeneous plants in adapted and unadapted environments and of genotype-environment interaction.

SUMMARY

1. The homozygous dominant genotype (*DD*) was compared with the heterozygous genotype (*Dd*) in a genetically heterozygous but homogeneous background. Eight

inbred lines and their sub-strains characterized by a homozygous recessive mutant were crossed onto three testers that had been obtained by doubling monoplolds. The crosses were grown in eight separate randomized block designs. Pertinent orthogonal comparisons were calculated in the analyses. Sampling variances and ratios of such variances were presented.

2. Without exception there was no evidence of overdominance for yield. On other attributes occasionally the heterozygotes exceeded the homozygous dominants. Variance ratios indicated that genetic homogeneity was not a valid assumption for all mutants or all attributes. Causes of heterozygosity in inbred lines were discussed.

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