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Epistasis in maize (*Zea mays* L.)

2. Genetic effects in crosses among early flint and dent inbred lines determined by three methods

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Summary. Three flint and three dent maize (*Zea mays* L.) inbred lines, their possible F_1 crosses, F_2 and backcross progenies, and all possible three-way crosses were evaluated in a three-year experiment for yield, ear moisture, and plant height. The purpose was to estimate genetic parameters in European breeding materials from (i) generation means analysis, (ii) diallel analysis of generation means, and (iii) analysis of F_1 and three-way cross hybrids. Method (i) was based on the F_∞-metric model and methods (ii) and (iii) on the Eberhart-Gardner (1966) genetic model; both models extended for heterotic maternal effects.

Differences among generation means for yield and plant height were mainly attributable to dominance effects. Epistatic effects were significantly different from zero in a few crosses and considerably reduced heterosis in both traits. Additive × additive and dominance × dominance effects for yield were consistently positive and negative, respectively. Significant maternal effects were established to the advantage of generations with a heterozygous seed parent. In the diallel analysis, mean squares for dominance effects were greater than for additive effects for yield and plant height but smaller for ear moisture. Though significant for yield and plant height, epistatic variation was small compared to additive and dominance variation. Estimates of additive × additive epistasis for yield were significantly negative in 11 of 15 crosses, suggesting that advantageous gene combinations in the lines had been disrupted by recombination in the segregating generations. The analysis of hybrids supported the above findings regarding the analysis of variance. However, the estimates of additive × additive epistasis for yield were considerably smaller and only minimally correlated with those from the diallel analysis. Use of noninbred materials as opposed to materials with different levels of inbreeding is considered the main reason for the discrepancies in the results.

Key words: Epistasis – Heterosis – Maternal effects – Generation means analysis – *Zea mays* L.

Introduction

Knowledge about the nature of gene action involved in conditioning the expression of economically important traits is fundamental to optimizing breeding programs. Two approaches based on first-degree statistics have been used to evaluate the importance of epistatic effects in polygenically inherited characters: generation means analysis and comparisons among different types of hybrids.

Generation means analysis was introduced by Anderson and Kempthorne (1954) and Hayman (1958) to provide information on additive, dominance and digenic epistatic effects in crosses between specific pairs of lines. Studies in maize (*Zea mays* L.) employing this approach detected high frequencies of significant estimates for each epistatic parameter for grain yield and other agronomic characters (Hallauer and Miranda 1981). An extension of this approach is to perform a joint analysis on the generation means derived from a diallel or factorial mating design (Gardner and Eberhart 1966; Stuber and Moll 1969).

Bauman (1959) and other researchers utilized comparisons among various types of hybrids for evaluating net epistasis (for review, Melchinger 1984). These studies indicated that significant epistatic effects exist for certain hybrid combinations. Regarding the average performance level, experiments with unselected lines showed negligible differences among different types of hybrids, whereas experiments with elite inbred lines established epistatic effects to the advantage of single crosses.

Experimental evidence from both approaches suggests that additive and dominance effects are of major importance in the inheritance of yield and other agronomic traits. However, estimates of epistatic effects were generally greater and more frequently significant in generation means studies than in comparisons among different hybrid types. These inconsistencies may occur firstly from the different experimental approaches (i.e., investigating materials with different levels of inbreeding as opposed to noninbred materials) and from differences in the underlying genetic model.

The impetus for the present research was to investigate epistasis in both types of materials established from the same set of lines by (i) generation means analysis, (ii) diallel analysis, and (iii) analysis of different hybrid types. A similar study was conducted by Moreno-Gonzales and Dudley (1981), who presented results from the three methods. However, to permit a more direct comparison, the same genetic model should be applied to each approach. Such a synoptic analysis is possible using the Eberhart and Gardner (1966) genetic model.

Results from the comparison between single and three-way crosses have been reported elsewhere (Melchinger et al. 1986). The present investigation has four primary objectives: 1) to estimatic genetic parameters by generation means analysis in European materials generated from a diallel cross; 2) to assess the bias in the estimates arising from maternal effects; 3) to measure the variation associated with maternal, additive, dominance, and additive × additive epistatic effects in the diallel by applying an extended version of the Eberhart-Gardner model; and 4) to compare the estimates of epistatic effects from the latter analysis with those obtained from analyzing the single and three-way cross data.

Materials and methods

Materials

The materials studied, the design of field experiments, and the measurements taken have been previously described (Melchinger et al. 1985, 1986). Briefly, six unrelated inbred lines, three being of flint (DS 8, DS 10, DS 13), and three of dent (CO 125, DS 503, DS 513) origin, were used to establish the experimental material of this study. All lines designated by DS were developed by F. W. Schnell in West Germany from European flint and North American dent materials, respectively. CO 125 is a Canadian line extensively used in the production of commercial hybrids in West Germany and France. The lines were known to combine earliness with good general combining ability for grain yield.

The six lines were mated in a diallel series to develop the 15 possible F_1 crosses in both reciprocal forms, their selfed progenies (F_2), and their first backcrosses to both parents (BC 1 and BC 2) each in both reciprocal forms F_1P and PF_1, respectively. In addition, the 60 possible three-way crosses (3 W) were produced employing the inbred line as seed parent. To minimize seed quality differences, all tested materials were produced in the same year (1979).

Yield trials

The study was conducted at Stuttgart-Hohenheim (FRG) in 1980, 1981, and 1982. The experimental design for the F_1, F_2, BC 1, and BC 2 generations, respectively, was a split plot with crosses comprising the whole plots and the two reciprocal forms of a cross comprising the subplots. The whole plots (15 crosses and one check of the same degree of inbreeding) were arranged as a quadruple 4×4 lattice for each generation. The 60 3 W crosses and four checks were evaluated in a 8×8 quadruple lattice. The six lines were tested in a randomized complete block design (RCB) with 24 replicates.

The six experimental designs were not arranged separately in the field but organized in six groups of adjacent blocks within each replication to minimize the effects of soil heterogeneity on generation means. Four groups each consisted of four incomplete blocks: one block for the F_1, F_2, BC 1, and BC 2 generations, respectively; each group comprised progenies from the same four crosses. The two remaining groups included the eight incomplete blocks from the 3 W and six complete blocks from the RCB design of the lines, respectively. Apart from this restriction, ordering of complete or incomplete blocks within each group and entries within blocks was random. Each block was bordered by buffer rows of appropriate vigor levels.

The experimental unit in all designs was a one-row plot with 20 plants. Average plant density for all groups of materials was 7.7 plants/m^2. Measurements of four quantitative traits were studied: silking date (days after sowing), plant height (cm) measured from ground level to the lowest tassel branch, ear moisture (%) at harvest, and grain yield (q/ha) adjusted to 15.5% moisture. All experiments were harvested by hand.

Statistical procedures

Ordinary lattice analyses of variance were performed with the data gathered from the 3 W's and the nonhomozygous generations in each year, reciprocals being analyzed separately. Adjusted means and effective error mean squares from the lattice analyses as well as means and error mean squares from the RCB analyses were obtained for further use in the quantitative genetic analyses.

Generation means analysis

The F_∞-metric genetic model (Mather and Jinks 1982, p 83), extended by a term for maternal effects, was fitted to the generation means for each cross. Gamble's notation (1962a), originally referring to parameters in the F_2-metric, was used for convenience. The formulae for the genotypic means of the various generations are:

$$
\begin{array}{llllllll}
P_1 & = m & & + a & & + aa & & \\
P_2 & = m & & - a & & + aa & & \\
F_1 & = m & & & + d & & & + dd \\
F_2 & = m & + M & & + d/2 & & & + dd/4 \\
BC1\,(F_1P) & = m & + M & + a/2 & + d/2 & + aa/4 & + ad/4 & + dd/4 \\
BC1\,(PF_1) & = m & & + a/2 & + d/2 & + aa/4 & + ad/4 & + dd/4 \\
BC2\,(F_1P) & = m & + M & - a/2 & + d/2 & + aa/4 & - ad/4 & + dd/4 \\
BC2\,(PF_1) & = m & & - a/2 & + d/2 & + aa/4 & - ad/4 & + dd/4
\end{array}
$$

where P_1 and P_2 are the inbred parents; m is the mean of all inbred lines derived from the cross $P_1 \times P_2$; M is the maternal effect attributed to a heterozygous seed parent; a, d, aa, ad, and dd denote the pooled additive, dominance, additive × additive, additive × dominance, and dominance × dominance effects, respectively.

Least squares regression techniques were employed to obtain the parameter estimates and the corresponding standard errors as outlined by Mather and Jinks (1982, p 162).

Significance of the estimates was determined by the usual t-test.

Diallel analysis

An alternative analysis of generation means derived from a diallel series was suggested by Eberhart and Gardner (1966). Their model, which accounts for additive × additive epistatic effects, was extended by Carbonell et al. (1983) to include "heterotic" maternal effects. The complete genetic model for all generations of the diallel can be written as follows:

$$\begin{aligned}
&\mathrm{P_i} &&= \mu &&+ (a_i + aa_i)\\
&\mathrm{F_1\,(i \times j)} &&= \mu &&+ \tfrac{1}{2}(a_i + aa_i) + \tfrac{1}{2}(a_j + aa_j) + d_{ij} + aa_{ij}\\
&\mathrm{F_2\,(i \times j)} &&= \mu + M_{ij} &&+ \tfrac{1}{2}(a_i + aa_i) + \tfrac{1}{2}(a_j + aa_j) + \tfrac{1}{2}d_{ij} + aa_{ij}\\
&\mathrm{BC\,1\,(i \cdot j \times i)} &&= \mu + M_{ij} &&+ \tfrac{3}{4}(a_i + aa_i) + \tfrac{1}{4}(a_j + aa_j) + \tfrac{1}{2}d_{ij} + \tfrac{3}{4}aa_{ij}\\
&\mathrm{BC\,1\,(i \times i \cdot j)} &&= \mu &&+ \tfrac{3}{4}(a_i + aa_i) + \tfrac{1}{4}(a_j + aa_j) + \tfrac{1}{2}d_{ij} + \tfrac{3}{4}aa_{ij}\\
&\mathrm{BC\,2\,(j \cdot i \times j)} &&= \mu + M_{ij} &&+ \tfrac{1}{4}(a_i + aa_i) + \tfrac{3}{4}(a_j + aa_j) + \tfrac{1}{2}d_{ij} + \tfrac{3}{4}aa_{ij}\\
&\mathrm{BC\,2\,(j \times j \cdot i)} &&= \mu &&+ \tfrac{1}{4}(a_i + aa_i) + \tfrac{3}{4}(a_j + aa) + \tfrac{1}{2}d_{ij} + \tfrac{3}{4}aa_{ij}
\end{aligned}$$

$\sum_i a_i = 0$, $\sum_i aa_i = 0$, where $i = 1, \ldots, n$ is the number of inbreds, μ is the mean of all inbred lines, and M_{ij} are the maternal effects. Detailed definitions of the parameters a_i and aa_i, d_{ij}, and aa_{ij}, denoting the pooled additive, additive × additive effects of line P_i, and dominance and additive × additive effects of the cross $P_i \times P_j$, are given by Eberhart and Gardner (1966). A link between the parameters of the F_∞-metric and those in the Eberhart-Gardner model was presented by Cockerham (1980).

Unfortunately, a_i and aa_i are completely confounded and cannot be estimated separately. Instead, the parameter

$$a_i^* = a_i + aa_i$$

was estimated.

As the parameters of this model are not orthogonal, four models were fitted sequentially:

Model	Parameters
1	μ, M_{ij}
2	μ, M_{ij}, a_i^*
3	$\mu, M_{ij}, a_i^*, d_{ij}$
4	$\mu, M_{ij}, a_i^*, d_{ij}, aa_{ij}$.

Standard least squares procedures were used to obtain the sums of squares due to regression. The combined analysis was computed using lattice-adjusted means and entry means (in the case of the parents) from each year. Additive × additive epistatic effects were estimated and the means of the F_1 crosses were predicted from the F_1, F_2, BC, and parent lines data.

Analysis of single and three-way crosses

The model equation for the single crosses, being identical to the F_1 crosses, was given in the foregoing section. The model for the genotypic value of the three-way cross $i \times j \cdot k$ given by Eberhart and Gardner (1966) is

$$\begin{aligned}
3\,W_{i \times j \cdot k} = \mu + a_i^*/2 + (a_j^* + a_k^*)/4 + (d_{ij} + d_{ik})/2\\
+ (aa_{ij} + aa_{ik})/2 + aa_{ij}/4,
\end{aligned}$$

using the above notation. Proceeding similarly as in the diallel analysis, this model was fitted to the F_1 and 3 W data to estimate the additive × additive epistatic effects and to predict the performance of the F_1 crosses.

All necessary computations in the three methods were accomplished using the REGRESS directive of Genstat (Numerical Algorithm Group 1977).

Results

Generation means analysis

Generation means were averaged over crosses within the FLINT × FLINT, FLINT × DENT, and DENT × DENT groups. The averages of the FLINT crosses were lower than of the DENT crosses for all traits (Table 1). The FLINT × DENT crosses showed higher generation means that the two other groups for grain yield and intermediate values for the other traits with only one exception. Heterosis was greatest for grain yield, averaging 135% over all crosses. In comparison with the inbred parents, the F_1 crosses silked earlier and had a lower ear moisture.

The F_2 generation means were significantly ($P < 0.05$) smaller than the BC(F_1P) means in all groups for grain yield. The comparison of BC(F_1P) with BC(PF_1) showed consistently higher yields when the F_1 was used as the seed parent. Both the F_2 and BC(PF_1) yields were smaller than the mean of the F_1 and midparent performances.

Estimates of m were significantly different from zero for grain yield in all 15 crosses (Table 2) and showed lower values than the midparent performances. Maternal effects (M) were consistently positive and significantly different from zero for eight crosses. Additive (a) and additive × dominance effects (ad) were small in most crosses compared with the other gene effects. The sign of both parameters is meaningless because it depends upon which parent is designated as P_1 and which as P_2. Dominance effects (d) were significant in all crosses. Estimates were slightly greater in the FLINT × DENT crosses than in the other groups. Additive × additive effects (aa) were consistently positive and dominance × dominance effects (dd) correspondingly negative. The two categories of effects were similar in magnitude being approximately one fifth of the estimates of d. Estimates of aa varied little among crosses whereas estimates of dd showed a greater range. On the average, epistatic effects (aa, dd) reduced heterosis (h) by about 40%.

Maternal effects (M) were mostly negative for ear moisture (Table 3). Significant additive effects (a) were detected in nine crosses. Both dominance effects (d) and epistatic effects (aa, dd) showed no consistent sign, the former being significant in one cross.

Results for plant height (Table 4) were similar to those for grain yield. Dominance effects (d) were the most important gene effects, being significant in ten crosses. Dominance × dominance epistasis (dd) generally prevailed over other types (aa, ad). Heterosis (h) was considerably diminished by positive aa and negative dd effects in most crosses.

Table 1. Generation means and heterosis values averaged over crosses within groups and over years for four agronomic traits

Generation	FLINT crosses ($n=3$)[a]	FLINT×DENT crosses ($n=9$)	DENT crosses ($n=3$)	FLINT crosses ($n=3$)	FLINT×DENT crosses ($n=9$)	DENT crosses ($n=3$)
	Grain yield (q/ha)			Ear moisture (%)		
F_1[b]	75.5±2.1[e]	85.9±1.2	80.6±2.1	41.07±0.31	41.61±0.18	42.06±0.31
F_2[b]	52.3±1.6	57.9±0.9	56.8±1.6	42.51±0.33	42.38±0.19	42.58±0.33
BC (F_1P)[c]	55.7±1.5	62.7±0.9	62.3±1.5	42.48±0.29	42.51±0.17	42.62±0.29
BC (PF_1)[c]	54.0±1.6	59.5±0.9	57.5±1.6	42.67±0.27	43.05±0.16	43.25±0.27
Parents	32.6±2.4	35.3±1.7	38.0±2.4	44.33±0.70	44.50±0.50	44.68±0.70
Heterosis (%)[d]	132	143	112	−7	−6	−6
	Plant height (cm)			Silking date (days)		
F_1[b]	179.6±2.6	187.6±1.5	190.8±2.6	83.22±0.26	84.56±0.15	86.16±0.26
F_2[b]	148.6±2.5	154.3±1.5	156.5±2.5	83.82±0.30	85.35±0.17	87.10±0.30
BC (F_1P)	150.0±2.3	155.9±1.3	160.5±2.3	84.03±0.34	85.45±0.20	87.40±0.34
BC (PF_1)	149.7±2.4	157.0±1.4	161.4±2.4	84.30±0.33	85.80±0.19	87.50±0.33
Parents	110.6±3.5	119.2±2.5	127.7±3.5	88.09±0.66	90.22±0.38	92.35±0.66
Heterosis (%)	62	57	49	−6	−6	−7

[a] Number of crosses in the respective group; [b] Averaged over reciprocal crosses; [c] Averaged over backcrosses to both parents (BC1 and BC2); [d] Heterosis is measured as 100 (F_1-parents)/parents; [e] Standard error associated with each mean

Table 2. Gene effects estimated from generation means analyses for grain yield (q/ha)

Origin	Cross	Gene effects[a]							
		m	M	a	d	aa	ad	dd	h[b]
FLINT	DS8×DS10	21.3	2.4	−0.3	66.2	12.3	−4.4	−14.1	39.7
	DS8×DS13	19.8	0.2	1.3	78.7	12.2	10.4	−23.8	42.8
	DS10×DS13	15.6	2.4	1.6	65.7	16.7	17.2	−3.0	46.0
FLINT×DENT	DS8×CO125	20.1	6.7	−5.0	84.0	18.2	1.8	−5.8	60.0
	DS8×DS503	20.2	0.1	−5.2	93.5	18.3	−0.2	−25.6	49.7
	DS8×DS513	16.0	5.1	3.0	74.2	14.3	19.9	−7.6	52.3
	DS10×CO125	21.3	5.4	−4.6	73.1	17.2	−0.8	−3.4	52.5
	DS10×DS503	16.1	0.0	−4.8	112.5	22.7	8.3	−44.6	45.3
	DS10×DS513	12.2	4.6	3.4	76.8	18.4	5.9	−8.7	49.7
	DS13×CO125	20.0	4.9	−6.3	74.4	17.1	−7.0	−3.3	54.1
	DS13×DS503	12.5	1.0	−6.5	85.4	24.6	−7.1	−13.8	46.9
	DS13×DS513	10.1	1.1	−1.7	79.5	18.9	−2.4	−16.0	44.6
DENT	CO125×DS503	14.4	5.3	−0.2	97.8	29.0	8.5	−26.9	41.9
	CO125×DS513	15.0	4.9	8.0	77.8	20.2	18.3	−15.2	42.4
	DS503×DS513	18.4	4.2	8.2	62.8	17.0	10.7	−2.4	43.4
	Mean	16.9	3.2	–[c]	80.2	18.5	–[c]	−14.3	47.4
	S.D.[d]	3.6	2.3	–[c]	13.2	4.4	–[c]	11.8	5.5
	S.E.[e]	5.1	1.8	2.9	30.0	12.3	7.9	18.2	4.7
	N[f]	15	8	4	15	2	3	1	15

[a] For definition of effects see "Materials and methods"

[b] h refers to heterosis, h = d − aa + dd

[c] Not calculated, since the sign of the estimate for a cross depends upon which parent is taken as P_1

[d] Standard deviation of estimates among crosses

[e] Standard error associated with each estimate

[f] Number of crosses out of 15 showing estimates significantly different from zero at the 0.05 level of probability

Table 3. Gene effects estimated from generation means analyses for ear moisture (%)

Origin	Cross	Gene effects[a]							
		m	M	a	d	aa	ad	dd	h[b]
FLINT	DS8×DS10	43.2	0.5	1.2	-1.9	1.5	-4.7	0.3	-3.1
	DS8×DS13	46.5	-0.6	1.2	-7.5	-1.7	-0.8	1.2	-4.6
	DS10×DS13	43.9	-0.5	-0.1	-1.6	-0.1	1.5	-0.7	-2.1
FLINT×DENT	DS8×CO125	43.8	-0.9	3.0	-2.7	-0.9	-3.3	-0.7	-2.5
	DS8×DS503	41.6	-0.5	2.0	3.8	2.3	2.0	-3.6	-2.1
	DS8×DS513	49.0	-0.9	-3.2	-7.9	0.1	0.7	3.4	-4.6
	DS10×CO125	41.2	-1.0	1.8	0.5	0.5	3.3	-2.0	-2.1
	DS10×DS503	43.7	-0.5	0.8	-2.3	-1.1	1.1	-0.1	-1.3
	DS10×DS513	43.2	-0.4	-4.4	3.2	4.7	5.2	-3.3	-4.9
	DS13×CO125	40.1	-0.4	1.8	0.6	1.7	0.6	-2.3	-3.4
	DS13×DS503	44.4	-0.3	0.9	-1.8	-1.6	1.2	-1.2	-1.3
	DS13×DS513	49.0	0.1	-4.3	-10.3	-1.1	4.7	5.3	-3.9
DENT	CO125×DS503	38.5	-0.7	-1.0	4.5	2.4	-1.7	-3.9	-1.9
	CO125×DS513	50.9	-1.4	-6.1	-13.3	-4.8	-0.8	5.3	-3.2
	DS503×DS513	44.2	0.2	-5.2	0.5	2.9	-0.3	-0.4	-2.8
	Mean	44.2	-0.5	–[c]	-2.4	0.3	–[c]	-0.2	-2.9
	S.D.[d]	3.4	0.5	–[c]	5.2	2.3	–[c]	2.9	1.2
	S.E.[e]	2.8	0.5	0.8	6.0	2.6	2.0	3.6	1.0
	N[f]	15	2	9	1	0	3	0	11

[a] For definition of effects see "Materials and methods"

[b] h refers to heterosis, $h = d - aa + dd$

[c] Not calculated, since the sign of the estimate for a cross depends upon which parent is taken as P_1

[d] Standard deviation of estimates among crosses

[e] Standard error associated with each estimate

[f] Number of crosses out of 15 showing estimates significantly different from zero at the 0.05 level of probability

Table 4. Gene effects estimated from generation means analyses for plant height (cm)

Origin	Cross	Gene effects[a]							
		m	M	a	d	aa	ad	dd	h[b]
FLINT	DS8×DS10	120.6	-1.6	-6.5	57.0	-10.5	4.5	-0.8	66.7
	DS8×DS13	91.7	1.8	-4.0	143.4	15.8	22.7	-61.1	66.4
	DS10×DS13	102.5	0.6	2.6	95.9	11.6	21.5	-10.4	73.9
FLINT×DENT	DS8×CO125	110.7	1.1	-20.1	132.7	13.9	8.7	-42.7	76.1
	DS8×DS503	109.7	0.4	-15.1	95.9	9.0	5.0	-15.1	71.8
	DS8×DS513	110.7	-3.4	-0.1	88.9	-7.0	18.0	-24.7	71.2
	DS10×CO125	106.3	-4.0	-14.5	129.3	24.8	6.0	40.8	63.7
	DS10×DS503	139.6	-4.8	-8.6	66.0	-14.3	28.9	7.0	87.4
	DS10×DS513	91.5	-1.0	6.4	116.5	18.7	8.0	-28.2	69.6
	DS13×CO125	117.6	1.0	-17.0	95.0	10.9	-11.4	-25.5	58.7
	DS13×DS503	133.8	0.2	-11.2	30.0	-11.1	-2.6	19.8	60.9
	DS13×DS513	95.7	-0.1	3.9	106.5	12.0	-0.1	-37.9	56.6
DENT	CO125×DS503	108.7	-4.0	5.8	146.5	31.0	7.8	-49.2	66.2
	CO125×DS513	128.6	-1.0	20.9	48.9	-3.9	11.2	5.7	58.5
	DS503×DS513	97.3	2.5	15.0	115.9	21.6	-4.3	-29.8	64.3
	Mean	111.0	-0.8	–[c]	98.7	8.2	–[c]	-23.8	66.7
	S.D.[d]	14.8	2.3	–[c]	35.0	14.2	–[c]	23.0	8.0
	S.E.[e]	7.8	4.0	4.3	45.5	20.9	11.9	26.8	6.3
	N[f]	15	0	7	10	0	1	1	15

[a] For definition of effects see "Materials and methods"

[b] h refers to heterosis, $h = d - aa + dd$

[c] Not calculated, since the sign of the estimate for a cross depends upon which parent is taken as P_1

[d] Standard deviation of estimates among crosses

[e] Standard error associated with each estimate

[f] Number of crosses out of 15 showing estimates significantly different from zero at the 0.05 level of probability

Table 5. Mean squares and coefficient of determination (R^2) from the diallel analysis of the generation means after the Eberhart-Gardner (1966) model for grain yield, ear moisture, and plant height

Source of variation	d.f.	Mean squares		
		Grain yield (q/ha)	Ear moisture (%)	Plant height (cm)
Entries	125	1,056**	29.3**	2,408**
Maternal (M) effects	15	384**	43.2**	1,010**
Additive (a^*) effects	5	2,042**	468.0**	9,880**
Dominance (d) effects	15	7,527**	35.3**	15,388**
Epistatic (aa) effects	15	66**	1.2	42*
Deviations	75	29**	1.6*	66*
Years × Entries	250	43**	4.2**	89**
Years × M effects	30	48**	4.9**	79**
Years × a^* effects	10	243**	16.4**	501**
Years × d effects	30	127**	18.0**	234**
Years × aa effects	30	24**	0.9**	19
Years × deviations	150	17**	1.1**	48**
Pooled error	1,137	7	0.3	18
R^2 (μ, M, a^*, d)		97.6	96.1	98.1
R^2 (μ, M, a^*, d, aa)		98.4	96.6	98.3

*,** Significant at the 0.05 and 0.01 levels of probability, respectively

Diallel analysis

Analysis of generation means utilizing the Eberhart-Gardner model showed significant variation due to maternal (M_{ij}), additive (a_i^*) and dominance effects (d_{ij}) for each trait (Table 5). Additive × additive effects (aa_{ij}) and deviations from the complete model were significant except for ear moisture and plant height, respectively. The coefficients of determination (R^2) for model 3 (μ, M_{ij}, a_i^*, d_{ij}) exceeded 0.96 for all traits and increased by less than 0.01, extending the model to additive × additive effects (Model 4). Interactions of all genetic effects with environments were significant except for epistatic effects of plant height.

Observed and predicted F_1 performances showed minimal differences ($Y - \hat{Y}^I$) for grain yield and plant height (Table 6). The estimates of the additive × additive effects ($\widehat{aa}_{ij}$) were significantly negative in 11 crosses for grain yield and significantly positive in six crosses for plant height.

Analysis of single and three-way crosses

Epistasis estimates determined according to Bauman's (1959) method were reported in an earlier paper (Melchinger et al. 1986). The analyses of variance indicated significant epistatic effects for all traits. The

Table 6. Observed means of F_1 crsses (Y), deviations between observed and predicted F_1 performance ($\hat{Y}$), and additive × additive epistatic estimates ($\widehat{aa}_{ij}$) for grain yield and plant height. The estimates were obtained from the combined analyses over years applying the Eberhart-Gardner (1966) model to material I (different degrees of inbreedings: F_1, F_2, BC, P) and material II (noninbred: F_1, 3W); the respective estimates are denoted by $\hat{Y}^I$, $\widehat{aa}_{ij}^I$ vs. $\hat{Y}^{II}$, $\widehat{aa}_{ij}^{II}$

Origin	Cross	Grain yield (q/ha)					Plant height (cm)				
		Y	$Y-\hat{Y}^I$	$Y-\hat{Y}^{II}$	$\widehat{aa}_{ij}^I$	$\widehat{aa}_{ij}^{II}$	Y	$Y-\hat{Y}^I$	$Y-\hat{Y}^{II}$	$\widehat{aa}_{ij}^I$	$\widehat{aa}_{ij}^{II}$
FLINT	DS8 × DS10	73.4	-0.4	-0.8	-5.5	1.1	176.8	0.2	0.1	7.8**	-2.8
	DS8 × DS13	74.8	-0.9	-0.6	2.3	-6.0*	174.0	-2.3	0.1	20.8**	16.9**
	DS10 × DS13	78.3	-0.1	0.9	-15.1**	-5.4	187.9	-0.4	0.2	-6.0*	-5.5*
FLINT × DENT	DS8 × CO125	98.2	-0.1	-0.6	-16.4**	-1.8	200.6	-1.5	3.3	9.9**	12.4
	DS8 × DS503	88.2	-0.9	0.6	-3.8	-2.3	190.5	-0.5	1.2	-0.8	5.6*
	DS8 × DS513	82.6	-0.3	-0.2	-9.6**	5.4	174.9	-0.9	0.4	20.8**	27.8**
	DS10 × CO125	91.0	0.0	-2.6	-17.0**	-1.6	194.8	-1.4	-0.6	-2.0	-0.7
	DS10 × DS503	84.1	-1.6	1.2	3.4	3.2	212.7	0.3	-1.9	9.1**	-6.9*
	DS10 × DS513	80.3	-0.3	1.4	-13.1**	-2.6	179.8	-1.0	-0.4	-2.6	3.5
	DS13 × CO125	91.0	-0.1	-0.6	-15.3**	-6.1*	187.2	-1.0	-0.5	4.6	2.8
	DS13 × DS503	84.1	-0.5	0.6	-15.9**	0.9	183.6	0.7	1.8	0.3	14.5**
	DS13 × DS513	73.6	-0.7	1.1	-7.5*	-9.4**	164.3	-1.5	-1.5	12.3**	12.8**
DENT	CO125 × DS503	85.3	-1.0	-1.3	-13.7**	-1.9	205.9	-1.9	0.8	-0.9	14.9**
	CO125 × DS513	77.6	-0.6	0.1	-10.9**	3.6	183.2	0.3	-1.0	-0.2	12.4**
	DS503 × DS513	78.9	-0.1	0.6	-15.1**	-16.0**	183.4	-1.2	-0.9	-2.4	-1.3
	Mean	82.8	-0.5	0.0	-10.2**	-2.6**	186.6	-0.8	0.1	4.7**	6.7**

*,** Significantly different from zero at the 0.05 and 0.01 levels of probability, respectively

inclusion of additive × additive effects (aa_{ij}) in the model increased R^2 from 0.92 to 0.95 for grain yield, 0.95 to 0.98 for ear moisture, and 0.87 to 0.93 for plant height.

Deviations between the observed and predicted F_1 performances ($Y - \hat{Y}^{II}$) were negligible in both traits (Table 6). Significant $\widehat{aa}_{ij}$ estimates were found in 4 and 9 of the 15 crosses for grain yield and plant height, respetively.

Discussion

Generation means analysis

Since we employed an extended model that included heterotic maternal effects (M), our estimates of gene effects do not fully correspond to those from previous studies. Ignoring maternal effects leads to the same estimates for a, ad, dd and h. However, the other parameters may be biased as follows:

$$*m = m + 2M; \quad *d = d - 2M; \quad *aa = aa - 2M,$$

where *m, *d, and *aa denote the expectations of the estimates for m, d, and aa, respectively. The estimates of M for grain yield given in Table 2 indicate that this bias may be severe. With more favourable climatic conditions during the early vegetative development, however, heterotic maternal effects tend to be smaller (Melchinger et al. 1985).

Dominance effects (d) accounted for most of the performance differences between generation means for grain yield and plant height. This result concurs with previous investigations conducted in the U.S.A. with Corn Belt dent materials (Gamble 1962a, b; Darrah and Hallauer 1972; McConnell and Gardner 1979; Moreno-Gonzalez and Dudley 1981). Estimates of parameter d were usually several times larger than those of parameter a. However, the importance of the various types of gene action cannot be inferred from the relative magnitude of the parameter estimates even though such conclusions have sometimes erroneously been drawn in literature. Since the method only permits pooled gene effects to be estimated, cancelling positive and negative effects may impair the parameters to a different degree.

Estimates of epistatic effects for grain yield reported here were similar to those of Gamble (1962a) and McConnell and Gardner (1979). In accordance with our results, but less consistently so, these authors and Darrah and Hallauer (1972) established mostly positive aa and negative dd effects. Moreno-Gonzalez and Dudley (1981) found small aa effects and fairly heterogeneous estimates of dd. On account of larger standard errors, the relative number of crosses showing significant epistatic effects was lower in the present investigation than in previous reports for all traits. In conclusion, reduction of heterosis by epistasis seems to be a frequent phenomenon for grain yield.

Diallel analysis

Evidence from the regression analyses (Table 5) substantiates the conclusion that variation due to dominance effects was much greater than due to additive effects for grain yield and plant height. In both traits, additive × additive epistatic effects contributed a small but significant amount to the genetic variation among entries. Data reported by Eberhart and Gardner (1966); McConnell and Gardner (1979); and Moreno-Gonzalez and Dudley (1981) showed similar ratios among the mean squares of the various genetic effects for grain yield.

Caution must be observed when assessing the relative importance of additive, dominance, and epistatic effects from the relative size of the corresponding mean squares (Stuber and Moll 1971). Because of the nonorthogonality of the parameters in the model, additive × additive and deviation mean squares contain only a portion of the epistatic effects. On the other hand, mean squares attributable to maternal effects, which were first fitted in the model, tend to be inflated by genetic effects.

The negative estimates of the aa_{ij} effects for grain yield (Table 6) suggest that certain advantageous gene combinations in the lines were disrupted by recombination in the F_2 and BC generations. Eberhart and Gardner (1966) also obtained predominantly negative aa_{ij} estimates, averaging at -8% of the F_1 yields compared with -12% reported here.

Analysis of single and three-way crosses

The mean of the aa_{ij} estimates obtained from the analysis of the hybrids amounted to -3.2% of the F_1 performance for grain yield (Table 6). The model equations show that this mean corresponds to four times the performance difference between the three-way and single crosses. As discussed by Melchinger et al. (1986), most experiments in literature have established a more distinct yield superiority of single crosses over three-way crosses than found in our materials.

Comparisons among methods of analyses

The results from the generation means analyses are not comparable with those of the other two methods because of the different genetic models employed. The F_∞-metric model accounts for all types of digenic epistasis but is restricted to pairs of parents. The Eberhart-Gardner (1966) model is confined to additive × additive epistasis but includes the whole set of parents. Moreover, the effects are defined differently thus explaining the opposing signs in the estimates of additive × additive epistasis.

The diallel analysis of generation means (material I) and the analysis of hybrids (material II) showed reasonable agreement for the results of the analyses of variance. In both approaches additive and dominance effects were the major source of variation in all traits. Material I, involving both inbred and noninbred generations, displayed increased genetic variation reducing the relative portion of epistatic variation compared to material II.

Considerable disagreement between both approaches occurred in the estimates of the additive × additive effects ($\widehat{aa}_{ij}$) for grain yield (Table 6). Estimates obtained from material I were much greater in size than those from material II and had a low correlation ($r = 0.20$). The two methods yielded estimates of similar size for plant height, being more highly correlated ($r = 0.56^*$).

Different kinds of limitations in the two approaches may explain the disparities in the results. In the generation means approach, plant development was delayed in the generations with a higher level of inbreeding as indicated by the silking date (cf. Table 1). Hence, the various generations may be subject to different environmental conditions during pollination and grain filling. Estimates of gene effects may, therefore, be biased with the response to different environmental patterns.

With a given plant density, different inbred generations may display different levels of competition within each level of vigor. Increased competition with the more vigorous generations for soil moisture, available plant nutrients, and solar irradiance is expected to result in a curvilinear convex heterozygosity-performance relation. However, our findings in Table 1 show a concave relationship suggesting that unequal competition was not a major disturbing factor among generations.

The various generations in the generation means approach have different seed parents. Estimates of nuclear-genetic effects may thus be confounded with cytoplasmic and maternal effects and their interactions with the genotype. By including reciprocals in the F_1, F_2, and BC generations and accounting for maternal effects in the model, consideration was taken to reduce this bias as much as possible.

In both the diallel analysis of generation means and the analysis of different hybrid types, estimates of additive × additive effects ($\widehat{aa}_{ij}$) may be biased by higher order epistatic effects and/or linkage. Presence of these factors was indicated by the significant deviation mean squares for grain yield. If there are alleles interacting in the homozygous state with other loci, inbreeding would enhance these kinds of epistatic interactions by increasing their frequency. This might explain the greater $\widehat{aa}_{ij}$ estimates for material I. Support for this argument comes from the fact that the $\widehat{aa}_{ij}$ estimates in the diallel analysis mainly depend on the performance difference between the F_2 and $BC(PF_1)$ generations, which is not disturbed by the foregoing three factors.

In conclusion, our findings corroborate the conflicting results between both approaches in the literature. It must no be overlooked, however, that the two approaches provide answers to different aspects of epistasis. The generation means approach deals primarily with the mode of gene action in quantitative traits particularly with the contributions of epistatic effects to heterosis in single cross hybrids. On the other hand, Bauman's approach is concerned with performance differences among different hybrid types and hence is more closely related to practical aspects of hybrid breeding.

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References

Anderson VL, Kempthorne O (1954) A model for the study of quantitative inheritance. Genetics 30:883–898

Bauman LF (1959) Evidence of non-allelic gene action in determining yield, ear height, and kernel row number in corn. Agron J 51:531–534

Carbonnell EA, Bell AE, Nyquist WE (1983) Sex-linked and maternal effects in the Eberhart-Gardner general genetics model. Biometrics 39:607–619

Cockerham CC (1980) Random and fixed effects in plant genetics. Theor Appl. Genet 56:119–131

Darrah LL, Hallauer AR (1972) Genetic effects estimated from generation means from diallel sets of maize inbreds. Crop Sci 12:615–621

Eberhart SA, Gardner CO (1966) A general model for genetic effects. Biometrics 22:864–881

Gamble EE (1962a) Gene effects in corn (*Zea mays* L.). 1. Separation and relative importance of gene effects for yield. Can J Plant Sci 42:339–348

Gamble EE (1962b) Gene effects in corn (*Zea mays* L.). 2. Relative importance for plant height and certain component attributes. Can J Plant Sci 42:349–358

Gardner CO, Eberhart SA (1966) Analysis and interpretation of the variety cross diallel and related populations. Biometrics 22:439–452

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames, Iowa

Hayman BI (1958) The separation of epistatic from additive and dominance variation in generation means. Heredity 12:371–390

Mather K, Jinks JL (1982) Biometrical genetics. Chapman and Hall, London

McConnell RL, Gardner CO (1979) Inheritance of several cold tolerance traits in corn. Crop Sci 19:847–852

Melchinger AE (1984) Effects of recombination in the parental populations on the means and combining ability variances in hybrid populations of maize (*Zea mays* L.). PhD Thesis, University of Stuttgart-Hohenheim

Melchinger AE, Geiger HH, Schnell FW (1985) Reciprocal differences in single-cross hybrids and their F_2 and backcross progenies in maize. Maydica 30:395–405

Melchinger AE, Geiger HH, Schnell FW (1986) Epistasis in maize (*Zea mays* L.). 1. Comparison of single and three-way cross hybrids among early flint and dent inbred lines. Maydica (in press)

Moreno-Gonzalez J, Dudley JW (1981) Epistasis in related and unrelated maize hybrids determined by three methods. Crop Sci 21:644–651

Numerical Algorithm Group (1977) Genstat, a general statistical program. Numerical Algorithm Group, Oxford, England

Stuber CW, Moll RH (1969) Epistasis in maize (*Zea mays* L.). 1. F_1 hybrids and their S_1 progeny. Crop Sci 9:124–126

Stuber CW, Moll RH (1971) Epistasis in maize (*Zea mays* L.). 2. Comparison of selected with unselected populations. Genetics 67:137–149