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Associations between quantitative traits and enzyme loci in the F2 population of a maize hybrid*

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Summary. Univariate and multivariate analyses were used to identify associations between eight enzyme marker loci and 11 quantitative traits of maize (*Zea mays* L.). The material analyzed included inbred lines Wf9 and Pa405, single-cross hybrid Wf9 × Pa405, and the F2 generation of the selfed single-cross hybrid. Each enzyme locus assayed was associated with at least one quantitative trait, and all quantitative traits were associated with genotypes at particular enzyme loci. Significant associations also were found between the level of heterozygosity per individual and nine of 11 quantitative traits. The total contribution to heterosis, for seed yield per plant, of genes linked with the eight enzyme loci, was 27% of the F2 mean and 18% of the difference in mean between the F1 hybrid and the inbred parents. Genes linked with *Glu1* accounted for nearly one third of the total dominance effect detected by the eight enzyme loci. The chromosome segments marked by loci with significant effects on seed yield were markedly overdominant. The large heterotic effects of chromosome segments marked by particular loci suggest that enzyme loci could be used to help transfer genes responsible for heterosis to inbred lines. We conclude that analyses of additional inbred lines, F1 hybrids, and F2 populations in more environments will halp identify specific associations between enzyme loci, or chromosome segments which they mark, and important agronomic traits.

Key words: *Zea mays* L. – Corn – Electrophoresis – Allozymes – Correlations – Heterosis – Population improvement

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Introduction

Since the early 1960's enzyme loci have been used to genetically characterize germplasm in 22 or more domestic plant species (Tanksley and Orton 1983).

Enzyme loci have been found to be very useful in studies of the effect of artificial and natural selection on allele and genotype frequency distributions in domestic populations of maize (*Zea mays* L.) (Kahler 1983a; Stuber and Moll 1972; Stuber et al. 1980), and barley (*Hordeum vulgare* L.) (Allard et al. 1972; Clegg et al. 1978). They have been used to study the mating system and genetic structure of populations in several economically important plant species including maize (Brown and Allard 1970; Kahler et al. 1984; Pollak et al. 1984), barley (Clegg et al. 1978; Kahler et al. 1975), tomatoes (*Lycopersicon pimpinellifolium*) (Rick et al. 1977), and Douglas fir trees (*Pseudotsuga menziesii*) [MIRB] (Shaw and Allard 1982). Other important uses include the identification of sexual (Gates and Buller 1979; Tanksley and Jones 1981) and somatic (Evans et al. 1980; Gamborg 1981; Wetter and Kao 1976) hybrids, genetic mapping and linkage studies (Nielson and Scandalios 1974; Nielson and Frydenberg 1971; Goodman et al. 1980), identification of associations or linkages between enzyme loci and genes controlling resistance to diseases (Medina-Filho 1980; Rick and Fobes 1974; Tanksley and Rick 1980), and identification of associations between enzyme loci and genes controlling quantitative traits (Copes 1975; El-Kassaby 1982; Gottlieb 1977; Hamrick and Allard 1975; Ledig et al. 1983; Mitton 1978; Mitton et al. 1981; Pollak et al. 1984; Stuber et al. 1982; Tanksley et al. 1981).

Most published reports on maize have focused on the effects of selection for increased grain yield on allozyme frequencies in experimental populations. Stuber et al. (1980) reported seedling changes in allele frequencies at eight enzyme loci in two maize populations, 'Jarvis Golden Prolific' and 'Indian Chief'; they concluded that the changes were due to selection for improved grain yield. In 1982, Stuber et al. reported results of a selection experiment in which allele frequencies at seven enzyme loci were manipulated in an unselected 'Jarvis Golden Prolific' population so that they were nearly identical to the frequencies in the improved population. They found that selection based solely on allele frequencies at seven enzyme loci resulted in a significant

increase in grain yield equivalent to one and one-half to two cycles of full-sib family selection for grain yield alone. They also found that ear number was increased significantly. All other reports concerned with the effect of selection for quantitative traits on allozyme frequencies in maize populations have led to the conclusion that drift, due to small population size, could account for the observed frequency changes (Brown 1971; Brown and Allard 1971; Kahler 1983a). Similarly, El-Kassaby (1982) did not find strong associations between enzyme genotypes and quantitative traits in a sample of progenies from 56 open-pollinated Douglas-fir trees.

Pollak et al. (1984) reported that four (*Acp1, Got1, Prx1,* and *Adh1*) of nine enzyme loci assayed in two experimental populations of 'Hays Golden' showed strong associations with particular quantitative traits. For example, locus *Acp1* was associated with grain yield, maturity, and leaf traits. Their results suggested that these four enzyme marker loci may be useful for selection studies in 'Hays Golden' germplasm. They concluded that "information from more populations and environments is necessary before the feasibility of using enzyme loci in plant improvement is known."

The objective of this study was to investigate associations between genotypes at enzyme marker loci and quantitative traits in an F2 population of a single-cross hybrid of maize.

Materials and methods

The materials included parental inbred lines Wf9 and Pa405, single-cross hybrid Wf9 × Pa405, the F2 population of the single-cross hybrid, and open-pollinated progeny of each adult F2 plant. In 1983, 4,000 random F2 seeds of the selfed F1 hybrid were over planted and thinned to one plant per each of 2,000 hills in the field. Plots were 30.5 m long, plants were spaced approximately 30.5 cm within rows, and rows were spaced approximately 102 cm apart.

A random sample of 460 competitive (plants that had two neighbors) adult F2 plants were tagged and measured for the following traits: plant height (plt, ht, cm), ear length (ear lgth, cm), ear diamter (ear diam, mm), number of seed rows per ear (seed rows/ear), cob diameter (cob diam, mm), seed length (seed lgth, mm), 100 seed weight (seed wt, g), seed yield of the primary ear (seed yld/ear, g), seed yield per plant (seed yld/plt, g), ear height (ear ht, cm), and stalk diameter (stalk diam, mm).

Eighteen random open-pollinated progeny from each of the 460 adult F2 plants were assayed by horizontal starch gel electrophoresis using the methods given by Kahler (1983a). The enzyme loci used for the study included: glutamate oxaloacetate transaminase 1 (*Got1*), malate dehydrogenase 2 (*Mdh2*), acid phosphatase 1 (*Acp1*), and 4 (*Acp4*), cathodal peroxidase 1 (*Prx1*), esterase 4 (*Est4*), β-glucosidase 1 (*Glu1*), and 6-phosphogluconate dehydrogenase 1 (*Pgd1*). Table 1 gives chromosomal locations for all of the loci except *Acp4* and *Prx1*. None of these eight loci are linked to each other (Goodman et al. 1980; Kahler 1983b). Allele and genotype designations for each locus have been summarized elsewhere (Kahler 1983b). The genotypic information from each progeny array was used to ascertain the maternal plant genotype for each of the eight enzyme marker loci. Progeny were assayed because adult plant samples did not give good resolution for these eight loci on starch gels. The probability of correct genotypic classification at each locus was ≧0.98 (Brown and Allard 1970).

Table 1. Chi-square goodness of fit to the 1:2:1 ratio of observed numbers of individuals with each genotype at an enzyme locus in the F2 population of single-cross hybrid Wf9 × Pa405

Enzyme locus	Chromosome location[a]	Genotype[b]			χ^2[2]	P[c]
		11	12	22		
Got1	3L	113	224	123	0.748	0.688
Mdh2	6L	113	245	102	2.483	0.289
Acp1	9	103	245	112	2.309	0.315
Acp4	?	100	250	110	3.913	0.141
Prx1	?	96	245	119	4.256	0.119
Est4	3S	103	231	126	2.309	0.315
Glu1	10L	123	240	88	9.287	0.010
Pgd1	6L	124	224	112	0.939	0.625

[a] Chromosome locations given by Goodman and Stuber (1983)

[b] Genotypes at each locus are coded 11, 12, 22 to represent the homozygote, heterozygote and homozygote, respectively. The original coded genotypes of parents Wf9 (P1), Pa405 (P2), and Wf9 × Pa405 (F1) at each locus are as follow:
P1 = *Got1-11, Mdh2-11, Acp1-33, Acp4-11, Prx1-33, Est4-22, Glu1-11, Pgd1-22*
P2 = *Got1-22, Mdh2-22, Acp1-11, Acp4-55, Prx1-22, Est4-55, Glu1-22, Pgd1-11*
F1 = *Got1-12, Mdh2-12, Acp1-13, Acp4-15, Prx1-23, Est4-25, Glu1-12, Pgd1-12*
Allelic and genotypic code numbers have been reported previously (Kahler 1983b)

[c] Probability of obtaining a greater chi-square value with a 1:2:1 ratio

Data analyses

Standard BMDP (Dixon et al. 1981) statistical programs were used to do multivariate analyses of variance and to calculate principal components. A multivariate analysis of variance was conducted for each locus to test the hypothesis that the three genotypes of each locus have identical means for all 11 quantitative trait variables. Rao's F approximation to Wilk's lambda distribution was used to calculate probabilities of getting more extreme multivariate mean differences among genotypes of enzyme loci than were observed (Dixon et al. 1981).

A one-way analysis of variance was conducted to test for the effects, of the three genotypes, of each locus on each quantitative trait and principal component. The sum of squares among genotypes was split into two parts resulting in two independent hypothesis tests, each with 1 and N-3 degrees of freedom, where N is the total numbers of plants. The first null hypothesis was that the mean of heterozygotes is not greater than the mean of homozygotes. This was regarded as a one-tailed test, because dominance in the positive direction is necessary to produce an F1 hybrid that exhibits heterosis. The difference between the mean of the heterozygotes and the mean of the homozygous plants was designated as d. This definition of d differs from the dominance difference between the mean of the heterozygous genotype and the midparental mean of the two homozygous genotypes, which also is designated as d by Falconer (1981) and others but is referred to as h by Mather and Jinks (1971) and Soller et al. (1976). In an F2 population with nearly equal frequencies of the two possible homozygotes, our d is expected to be a close approximation to

the dominance difference used in single-gene models of quantitative inheritance. The hypothesis that the sum of the d's for all loci was less than or equal to zero was subjected to a *t*-test. The standard error of the sum of d's was calculated as the square root of the sum of squares of all eight individual SE d's for each trait.

The second hypothesis test, based on the remaining sum of squares among genotypes, was that the two homozygotes of a marker locus do not differ with respect to a quantitative trait. This was regarded as a two-tailed test, because we were interested in detecting mean differences regardless of which homozygote happened to be "best". The difference between means of two homozygote genotypes was designated 2a. This is twice the statistic a for a locus used by Falconer (1981) and others to denote the mean effect of a homozygous genotype, relative to the midparental value. Falconer assigns genotypic values of a, −a, and d to the two homozygous genotypes and the heterozygote, respectively. The corresponding genotypic values, in the notation of Mather and Jinks (1971), are d, −d, and h.

To test the composite hypothesis that any one of the eight enzyme loci have different homozygote means for a trait, the quantity $t = 2a/SE\,2a$, for each locus, was postulated to have a normal distribution with a mean of zero. It follows that the sum of squares of t values for all eight loci should have a chi-square distribution with 8 degrees of freedom. The sum of the absolute deviations, |sum|, between homozygote means for all loci has an expected value of $E(\Sigma d) = [2n(n-1)\mathrm{Var}\,2a/\pi]^{1/2}$, where n is the number (8) of loci (Kendall and Stuart 1969). After correcting for this positive bias, the mean of the absolute deviations between homozygotes has a standard error which can be, and was, calculated from formula (10.40) of Kendall and Stuart (1969) to permit the use of a *t*-test of the hypothesis that the sum of the absolute differences in homozygote means, corrected for bias, is zero.

Prior to calculating values of d and 2a, all data on each quantitative trait were standardized to have a variance of 1.0 by dividing it by the standard deviation of the F2 data for the trait.

Results

Before analyses were conducted to evaluate associations between the enzyme marker loci and quantitative traits, a chi-square 'goodness of fit' test was conducted to determine whether genotypic frequencies in the F2 population fit the expected 1 : 2 : 1 Mendelian segregation ratio. Table 1 gives chi-square values for observed vs. expected genotype numbers at each of the eight enzyme loci assayed. Segregation distortion was found only for locus *Glu1*. We do not believe that the segregation distortion at this locus was due to contamination by seed mixing or outcrossing during selfing of the F1 hybrid, because the other seven loci segregated according to the expected ratio. The distorted segregation ratio for *Glu1* may have been due to either gametic or zygotic selection. Generally, results of the chi-square analyses showed that the population of adult plants from the selfed F1 hybrid Wf9 × Pa405 conformed to the expected F2 progeny ratio of 1 : 2 : 1.

Correlation among quantitative traits and principal components

Simple correlation coefficients (r) among quantitative traits are presented in Table 2. Forty-two of 55 (76%) trait pairs had correlation coefficients significant at the 1% probability level and an additional six of 55 (11%) trait pairs were significant at the 5% level. The greatest correlations (those with $r \geqq 0.40$) can be divided into two groups. The first group includes seed yield traits and ear lgth, ear diam, and cob diam. In the second group, plant ht was highly correlated with ear ht, cob diam significantly correlated with ear lgth, and stalk diam moderately correlated with seed yld.

Table 3 gives correlation coefficients between quantitative traits (standardized to have a variance of 1.0) and principal components, as well as the percentage of the variance in all traits accounted for by the principal components in the F2 population. The first principal component (PC1) accounted for 42%, and the first three principal components accounted for 69% of the total

Table 2. Matrix of correlation coefficients (r) between quantitative traits in the F2 population of single-cross hybrid Wf9 × Pa405

Quantitative trait		Quantitative trait										
		X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
Plt ht	X1	1.00										
Ear lgth	X2	0.16**	1.00									
Ear diam	X3	0.26**	0.44**	1.00								
Seed rows/ear	X4	0.12*	0.15**	0.39**	1.00							
Cob diam	X5	0.20**	0.44**	0.61**	0.43**	1.00						
Seed lgth	X6	0.15**	0.26**	0.54**	0.11*	0.25**	1.00					
Seed wt	X7	0.05	0.40**	0.49**	0.23**	0.26**	0.48**	1.00				
Seed yld/ear	X8	0.22**	0.79**	0.73**	0.25**	0.52**	0.46**	0.59**	1.00			
Seed yld/plt	X9	0.23**	0.74**	0.70**	0.24**	0.49**	0.43**	0.57**	0.95**	1.00		
Ear ht	X10	0.56**	−0.03	0.10	0.10*	0.13*	−0.03	−0.11*	0.01	0.05	1.00	
Stalk diam	X11	0.17**	0.39**	0.31**	0.12*	0.28**	0.26**	0.24**	0.40**	0.41	0.07	1.00

* $P = 0.05$ at $r = 0.11$ and ** $P = 0.01$ at $r = 0.15$

Table 3. Correlation coefficients between principal components and quantitative traits, and the percentage of the variance in traits accounted for by the principal components in the F2 population of single-cross hybrid Wf9 × Pa405

Trait[a]	Principal component[b]			% of variance explained by PC's
	1	2	3	
Seed yld/ear	0.935	−0.123	0.006	89.5
Seed yld/plt	0.913	−0.099	−0.016	84.4
Ear diam	0.840	0.079	0.109	72.4
Ear lgth	0.758	−0.140	0.033	59.4
Cob diam	0.670	0.251	0.341	62.8
Seed wt	0.627	−0.475	−0.389	77.0
Seed lgth	0.584	−0.178	−0.165	40.0
Stalk diam	0.514	0.034	−0.065	27.0
Ear ht	0.103	0.788	−0.414	80.3
Plt ht	0.329	0.674	−0.472	78.5
Seed rows/ear	0.330	0.479	0.713	84.7
% of variance explained	42.4	15.2	11.0	68.7

[a] See text for definitions of traits
[b] Correlation coefficients greater than 0.45 are underlined

variation among individuals in the set of traits. The first principal component was highly correlated with yield related traits and stalk diam. The second principal component was highly correlated with ear ht and plt ht, and the third principal component was highly correlated with number of seed rows per ear.

Associations between enzyme loci and quantitative traits

A one-way multivariate analysis of variance was conducted, for each locus, to test the hypothesis that the three genotypes of each locus have identical means for all 11 quantitative trait variables. Rao's F approximation to Wilk's lambda distribution was used to calculate probabilities of getting more extreme multivariate mean differences among genotypes of enzyme loci than were observed (Dixon et al. 1981; Pollak et al. 1984). The probabilities are given in the last row of Table 4. The three genotypes of *Got1*, *Prx1*, *Glu1*, and *Pgd1* differ, at the 1% level of significance, with respect to their means for one or more quantitative traits. Genotypes of *Mdh2* and *Acp4* have different means for one or more quantitative traits at the 5% level of significance.

Mean differences between heterozygotes and homozygotes for enzyme loci

The mean difference, d, between heterozygous and homozygous plants for each marker locus and quantitative trait, or principal component, is presented in Table 4. The standard error of d, SE d, and the probability of obtaining larger positive values of d by chance also are given in this table. A striking feature of the data is that there are far more positive than negative values of d, especially for yield related traits. This suggests that most, if not all, of the enzyme loci assayed are linked to or associated with genes which contribute to high seed yield in F1 hybrid plants.

Quantitative trait genes linked to *Got1*, *Est4*, and *Glu1* exhibited significant dominance effects, d, on the yield related first principal component (PC 1). For *Got1*, the significant d for PC 1 was reflected in significant dominance effects for seed yld/plt, seed yld/ear, ear lgth, ear diam and cob diam which are all highly correlated with PC 1. *Got1* dominance effects also were significant for plt ht and stalk diam. *Glu1* had a significant dominance effect on ear lgth and had very highly significant dominance effects on seed yld/ear and seed yld/plt. However, the quantitative trait genes linked with *Glu1* have little, if any, effect on plt ht and stalk diam. Hence, these genes appear to have a different spectrum of pleiotropic effects than the genes linked with *Got1*. *Est4* had significant dominance effects on only one of the original traits – ear diam. However, *Est4* dominance effects are significant at the 0.10 level for several other traits including seed rows/ear, cob diam, seed yld/ear, seed yld/plt, and stalk diam. *Acp1* had significant dominance effects on ear lgth and cob diam which are important determinants of seed yld/ear and seed yld/plt, and *Pgd1* had significant dominance effects on seed yld/plt, ear lgth, and cob diam.

Among the eight loci sampled in this study, only *Mdh2* and *Prx1* did not have significant effects on seed yield or seed yield determinants such as ear lgth and cob diam. The magnitude of dominance effects ranged up to 0.302 ± 0.092 F2 phenotypic standard deviations. This was the dominance effect of genes linked with *Glu1* on seed yld/ear and accounted for almost one third of the total dominance effect observed for seed yld/plt.

Mean differences between the two homozygous genotypes of enzyme loci

Table 5 gives the value 2a of the difference in F2 means between the two homozygotes at each enzyme locus, as well as its standard error (SE 2a) and the probability of observing a greater difference by chance alone. All eight loci exhibited highly significant effects on one or more traits. Significant differences occurred for stature, seed, and ear variables. No locus had homozygous genotypes which differed with respect to the yield related PC 1 or with respect to seed yld/ear

Table 4. Difference (d) in F2 means between heterozygotes and homozygotes, standard errors (SE d) of the mean difference, and the probability (Prob) of getting a greater difference than that obtained. Each variable was standardized to make its F2 phenotypic standard deviation equal to unity

Trait statistic	Enzyme locus								Total
	Got1	*Mdh2*	*Acp1*	*Acp4*	*Prx1*	*Est4*	*Glu1*	*Pgd1*	
Plt ht									
d[a]	0.192*	0.127	0.061	0.052	0.068	0.106	0.005	0.170*	0.781**
SE d	0.093	0.093	0.094	0.094	0.094	0.093	0.094	0.093	0.264
Prob	0.019	0.087	0.256	0.289	0.234	0.127	0.479	0.034	0.0015
Ear lgth									
d	0.185*	0.120	0.226**	−0.036	0.107	0.077	0.214**	0.142	1.034**
SE d	0.093	0.093	0.093	0.093	0.093	0.093	0.093	0.092	0.263
Prob	0.023	0.100	0.008	0.649	0.126	0.205	0.011	0.062	0.0000
Ear diam									
d	0.192*	0.011	0.041	0.058	0.028	0.164*	0.098	0.023	0.614**
SE d	0.093	0.093	0.094	0.094	0.094	0.093	0.093	0.094	0.264
Prob	0.019	0.453	0.332	0.268	0.382	0.039	0.148	0.404	0.0101
Seed rows/ear									
d	0.004	0.055	0.130	0.025	0.011	0.136	0.081	−0.014	0.429
SE d	0.093	0.094	0.093	0.094	0.094	0.093	0.092	0.093	0.264
Prob	0.483	0.279	0.082	0.394	0.454	0.072	0.189	0.558	0.0519
Cob diam									
d	0.204*	0.038	0.201*	0.028	0.130	0.121	0.014	0.223**	0.961**
SE d	0.093	0.094	0.092	0.094	0.094	0.093	0.093	0.093	0.264
Prob	0.014	0.343	0.015	0.381	0.082	0.097	0.439	0.008	0.001
Seed lgth									
d	0.108	−0.024	0.095	−0.066	−0.017	0.109	0.002	0.076	0.283
SE d	0.093	0.092	0.093	0.094	0.094	0.093	0.093	0.093	0.264
Prob	0.122	0.603	0.154	0.761	0.574	0.122	0.490	0.206	0.1414
Seed wt									
d	0.020	0.013	0.054	0.160*	−0.048	0.029	0.151*	−0.016	0.362
SE d	0.093	0.094	0.094	0.094	0.094	0.093	0.093	0.094	0.265
Prob	0.416	0.446	0.281	0.044	0.694	0.380	0.053	0.568	0.0856
Seed yld/ear									
d	0.175*	0.076	0.099	0.028	0.027	0.140	0.302**	0.136	0.984**
SE d	0.093	0.094	0.094	0.094	0.094	0.093	0.092	0.093	0.264
Prob	0.030	0.208	0.145	0.382	0.386	0.066	0.001	0.073	0.0001
Seed yld/plt									
d	0.173*	0.030	0.073	0.016	0.054	0.151	0.277**	0.162*	0.936**
SE d	0.093	0.094	0.094	0.094	0.094	0.093	0.093	0.093	0.264
Prob	0.031	0.375	0.218	0.434	0.281	0.053	0.001	0.041	0.0002
Ear ht									
d	0.139	0.049	−0.001	0.048	0.165*	0.118	0.090	0.069	0.676**
SE d	0.093	0.094	0.094	0.094	0.093	0.093	0.093	0.093	0.264
Prob	0.068	0.301	0.503	0.305	0.038	0.103	0.168	0.231	0.0052
Stalk diam									
d	0.227**	0.109	0.112	0.020	0.180*	0.043	0.086	0.085	0.862**
SE d	0.093	0.094	0.093	0.094	0.093	0.093	0.093	0.093	0.264
Prob	0.007	0.121	0.115	0.415	0.027	0.323	0.177	0.181	0.0005
PC 1									
d	0.214**	0.087	0.113	0.077	0.067	0.179*	0.221**	0.136	1.094**
SE d	0.093	0.094	0.094	0.094	0.094	0.093	0.093	0.093	0.265
Prob	0.011	0.178	0.114	0.207	0.237	0.027	0.009	0.072	0.0000
PC 2									
d	0.124	0.088	0.023	0.036	0.127	0.121	−0.018	0.097	0.599**
SE d	0.093	0.094	0.094	0.094	0.094	0.094	0.094	0.094	0.265
Prob	0.092	0.173	0.405	0.350	0.087	0.097	0.577	0.150	0.0120

(continued overleaf)

Table 4 (continued)

Trait statistic	Enzyme locus								Total
	Got1	*Mdh2*	*Acp1*	*Acp4*	*Prx1*	*Est4*	*Glu1*	*Pgd1*	
PC 3									
d	−0.073	−0.026	0.066	−0.035	−0.034	0.037	−0.013	−0.052	−0.130
SE d	0.094	0.094	0.093	0.094	0.094	0.094	0.093	0.093	0.264
Prob	0.784	0.609**	0.241	0.644	0.643	0.346	0.556	0.711	0.6892
Wilk's test[b]	0.001	0.023	0.073	0.031	0.009	0.122	0.001	0.000	

[a] All d values are in "F2 standard deviation units" instead of the original measurement units (e.g., g, cm, mm, etc.)
[b] Wilk's probabilities of getting more extreme multivariate differences among genotypes of each enzyme locus

Table 5. Differences (2a) in F2 mens between homozygotes, standard errors (SE 2a) of the mean difference, and the probability (Prob) of getting a greater difference than that obtained. The sum of absolute differences, |sum|, between homozygotes at all loci is given in the second to the last column. Also given in this column is the probability associated with a test of the hypothesis that none of the 8 loci affect each trait. In the last column is the sum of the absolute mean differences minus the sum of differences expected in the absence of genes affecting a trait. Also given in the last column are the standard error (SE 2a) and the probability (Prob) of obtaining a larger sum of differences by chance. Each trait was standardized so that its F2 phenotypic standard deviation was 1.0

Trait statistic	Enzyme locus								|Sum|	|Sum| minus bias
	Got1	*Mdh2*	*Acp1*	*Acp4*	*Prx1*	*Est4*	*Glu1*	*Pgd1*		
Plt ht										
2a[a]	0.215	0.164	0.128	0.254	0.171	0.333**	0.011	0.048	1.323*	0.518**
SE 2a	0.130	0.136	0.137	0.138	0.138	0.132	0.138	0.130	–	0.170
Prob	0.097	0.228	0.349	0.066	0.215	0.012	0.940	0.714	0.0377	0.0011
Earth lgth										
2a	0.010	0.013	0.043	0.355**	0.222	0.059	0.130	0.384**	1.218**	0.414**
SE 2a	0.130	0.137	0.136	0.137	0.137	0.133	0.137	0.129	–	0.169
Prob	0.936	0.923	0.751	0.010	0.105	0.656	0.342	0.003	0.0145	0.0073
Ear diam										
2a	0.267*	0.246	0.080	0.183	0.063	0.157	0.218	0.056	1.272	0.465**
SE 2a	0.129	0.137	0.137	0.138	0.138	0.132	0.137	0.131	–	0.170
Prob	0.039	0.072	0.558	0.184	0.646	0.236	0.113	0.666	0.0865	0.0031
Seed rows/ear										
2a	0.051	0.018	0.163	0.107	0.086	0.121	0.487**	0.347**	1.380**	0.575**
SE 2a	0.131	0.137	0.136	0.138	0.138	0.133	0.136	0.130	–	0.170
Prob	0.693	0.897	0.233	0.438	0.531	0.363	0.000	0.007	0.0035	0.0000
Cob diam										
2a	0.115	0.147	0.409**	0.052	0.065	0.066	0.230	0.167	1.251*	0.446**
SE 2a	0.130	0.137	0.135	0.138	0.138	0.133	0.137	0.130	–	0.170
Prob	0.376	0.282	0.002	0.706	0.639	0.618	0.094	0.199	0.0422	0.0043
Seed lgth										
2a	0.326**	0.459**	0.154	0.245	0.058	0.023	0.166	0.196	1.626**	0.822**
SE 2a	0.130	0.135	0.136	0.138	0.138	0.133	0.138	0.130	–	0.170
Prob	0.012	0.001	0.260	0.076	0.673	0.865	0.228	0.132	0.0013	0.0000
Seed wt										
2a	0.395**	0.076	0.108	0.087	0.227	0.161	0.066	0.040	1.161	0.353*
SE 2a	0.130	0.137	0.137	0.138	0.138	0.133	0.138	0.131	–	0.170
Prob	0.002	0.580	0.431	0.528	0.099	0.227	0.629	0.761	0.0591	0.0192
Seed yld/ear										
2a	0.103	0.038	0.019	0.037	0.044	0.074	0.110	0.080	0.505	−0.300
SE 2a	0.130	0.137	0.137	0.138	0.138	0.133	0.136	0.130	–	0.170
Prob	0.427	0.779	0.892	0.788	0.751	0.579	0.420	0.538	0.9714	0.9615

Table 5 (continued)

Trait statistic	Enzyme locus								|Sum|	|Sum| minus bias
	Got1	*Mdh2*	*Acp1*	*Acp4*	*Prx1*	*Est4*	*Glu1*	*Pgd1*		
Seed yld/plt										
2a	0.152	0.067	0.016	0.086	0.014	0.080	0.094	0.130	0.638	−0.168
SE 2a	0.130	0.137	0.137	0.138	0.138	0.133	0.137	0.130	–	0.170
Prob	0.243	0.624	0.908	0.535	0.921	0.548	0.493	0.318	0.8705	0.8383
Ear ht										
2a	0.186	0.115	0.167	0.207	0.349**	0.082	0.286*	0.115	1.508*	0.703*
SE 2a	0.130	0.137	0.137	0.138	0.136	0.133	0.137	0.130	–	0.170
Prob	0.153	0.402	0.222	0.133	0.010	0.535	0.037	0.378	0.0191	0.0000
Stalk diam										
2a	0.050	0.039	0.142	0.072	0.287	0.160	0.088	0.223	1.061	0.256
SE 2a	0.130	0.137	0.136	0.138	0.137	0.133	0.138	0.130	–	0.170
Prob	0.698	0.775	0.297	0.604	0.036	0.229	0.523	0.087	0.2162	0.0658
PC 1										
2a	0.153	0.117	0.058	0.008	0.191	0.053	0.132	0.138	0.851	0.043
SE 2a	0.130	0.137	0.138	0.138	0.138	0.133	0.137	0.131	–	0.170
Prob	0.239	0.393	0.673	0.955	0.167	0.692	0.337	0.290	0.6027	0.4013
PC 2										
2a	0.171	0.038	0.005	0.155	0.156	0.123	0.020	0.150	0.817	0.008
SE 2a	0.131	0.137	0.138	0.138	0.138	0.133	0.138	0.131	–	0.171
Prob	0.191	0.784	0.969	0.263	0.261	0.355	0.887	0.254	0.5934	0.4825
PC 3										
2a	0.148	0.082	0.313*	0.299*	0.285*	0.158	0.468**	0.280*	2.034**	1.227**
SE 2a	0.131	0.137	0.137	0.138	0.138	0.133	0.137	0.130	–	0.170
Prob	0.259	0.550	0.022	0.030	0.039	0.236	0.001	0.032	0.0001	0.0000

[a] All 2a values are in "F2 standard deviation units" instead of original measurement units (e.g., g, cm, mm, etc.)

and seed yld/plt. This is in striking contrast to the situation for dominance differences, where four of the eight loci were significant for seed yld/plt, PC 1 or both.

In the second to the last column of Table 5 are probabilities from tests of the composite hypothesis that any one or more of the eight enzyme loci have different homozygote means for a trait. These hypothesis tests are far from significant for seed yld/ear, seed yld/plt, and PC 1.

In the last column of Table 5 are sums of the absolute mean differences, corrected for bias, between homozygotes of all eight loci as well as their standard errors and the probabilities of observing greater differences by chance alone. These absolute sums are significant for most variables, but they are far from significant for seed yld/ear, seed yld/plt and the first principal component which is highly correlated with seed yield.

Spectrum of Got1 effects

Mean values for 11 quantitative traits of the two inbred parents Wf9 and Pa405, their F1 hybrid and the three observed genotypes at locus *Got1* in the F2 population from the F1 hybrid are listed in Table 6. These data demonstrate the relative performance of each *Got1* genotype for each trait. The mean performance of the F1 hybrid exceeded the mean performance of both parents for all traits except cob diam. Previously, we noted that *Got1* genotypes were associated with seven traits in the F2 population. The favored genotypes (those with the largest mean value for each trait) at each locus, which were significantly associated with a particular trait, are presented in Table 7. For example, genotypes at locus *Got1* were associated with particular traits in the following manner: *Got1-11* and *Got1-12* with larger ear diam, longer seeds and heavier seeds; *Got1-12* and *Got1-22* with taller plants; and *Got1-12* with higher seed yld/ear, higher seed yld/plt, and larger stalk diam.

Heterozygosity and performance

Means and standard errors for quantitative traits as functions of the number of loci for which plants were heterozygous are given in Table 8. These data were used to construct Fig. 1 which shows the relationships between the number of heterozygous loci per individual and ear lgth, seed yld/ear, and cob diam in the F2

Table 6. Mean (± standard error) values for 11 quantitative traits of inbreds Wf9 and Pa405, Wf9 × Pa405, and genotypes at locus *Got1* of F2 progeny from the F1 hybrid

Trait[a]	Wf9	Pa405	Wf9 × Pa405 F1	F2 Genotype[b]		
				11	12	22
Sample size	23	20	25	113	224	123
Plt ht	155.43 ± 1.56	144.33 ± 1.99	190.12 ± 1.35	169.68 ± 1.55	174.80 ± 1.08	173.30 ± 1.63
Ear lgth	15.07 ± 0.31	12.42 ± 0.48	20.71 ± 0.19	16.45 ± 0.23	16.89 ± 0.15	16.47 ± 0.23
Ear diam	40.30 ± 0.80	31.20 ± 0.11	47.00 ± 0.30	43.39 ± 0.32	43.58 ± 0.23	42.44 ± 0.34
Seed rows/ear	15.69 ± 0.49	12.28 ± 0.54	17.20 ± 0.26	16.34 ± 0.17	16.41 ± 0.14	16.45 ± 0.18
Cob diam	25.80 ± 0.40	19.10 ± 0.03	25.30 ± 0.02	23.78 ± 0.16	24.04 ± 0.12	23.57 ± 0.15
Seed lgth	9.52 ± 0.20	9.24 ± 0.25	12.20 ± 0.14	11.40 ± 0.10	11.33 ± 0.07	11.04 ± 0.10
Seed wt	18.14 ± 0.50	15.11 ± 0.40	22.82 ± 0.35	20.30 ± 0.30	19.73 ± 0.21	19.09 ± 0.25
Seed yld/ear	59.13 ± 5.90	52.47 ± 5.70	197.30 ± 5.42	117.91 ± 3.35	122.18 ± 2.31	114.28 ± 3.18
Seed yld/plt	59.13 ± 5.90	52.47 ± 5.70	235.47 ± 7.26	120.79 ± 3.38	124.12 ± 2.38	115.42 ± 3.06
Ear ht	72.04 ± 2.64	79.24 ± 1.63	89.24 ± 1.79	82.06 ± 1.45	82.75 ± 1.17	79.00 ± 1.37
Stalk diam	20.96 ± 0.38	21.81 ± 0.32	25.92 ± 0.35	25.07 ± 0.29	25.71 ± 0.21	24.91 ± 0.30

[a] See text for definitions of traits
[b] See Table 1 for genotypes of Wf9, Pa405, and Wf9 × Pa405

Table 7. A summary of quantiative traits associated with enzyme loci and favored genotypes at each locus

Enzyme locus	Associated trait	Favored genotype[a]
Got1	Plt ht	*Got1-12, Got1-22*
	Ear diam	*Got1-11, Got1-12*
	Seed lgth	*Got1-11, Got1-12*
	Seed wt	*Got1-11, Got1-12*
	Stalk diam	*Got1-12*
	Seed yld/ear	*Got1-12*
	Seed yld/plt	*Got1-12*
Mdh2	Seed lgth	*Mdh2-11, Mdh2-12*
Acp1	Ear lgth	*Acp1-13*
	Cob diam	*Acp1-13, Acp1-33*
Acp4	Ear lgth	*Acp4-15, Acp4-55*
Prx1	Ear ht	*Prx1-22, Prx1-23*
	Stalk diam	*Prx1-22, Prx1-23*
Glu1	Ear lgth	*Glu1-11, Glu1-12*
	Seed rows/ear	*Glu1-11, Glu1-12*
	Seed yld/ear	*Glu1-12*
	Seed yld/plt	*Glu1-12*
Est4	Plt ht	*Est4-25, Est4-55*
Pgd1	Ear lgth	*Pgd1-12, Pgd1-22*
	Seed rows/ear	*Pgd1-12, Pgd1-22*
	Cob diam	*Pgd1-12, Pgd1-22*
	Seed yld/plt	*Pgd1-12*

[a] Favored genotypes for each locus are those with the largest mean value for each trait. Favored genotypes are given only for cases where analysis of variance indicated a significant F value between genotypes at a locus and a quantitative trait

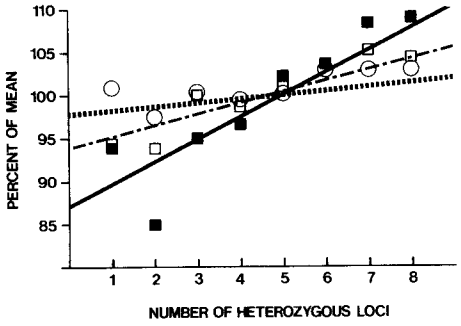

Fig. 1. Relationships between number of heterozygous loci per individual and mean ear length □, seed yield ■, and cob diameter ○ in the F2 population of Wf9 × Pa405. The percentage increase per heterozygous locus is b = 3.72 ± 0.95% for seed yield/ear (——), b = 1.76 ± 0.47% for ear length (-·-·-·-), and b = 0.86 ± 0.25% for cob diameter (- - - - -)

population of Wf9 × Pa405. The percentage increase in seed yld/ear is 3.72 ± 0.95% per heterozygous locus. The estimated difference in mean seed yld/ear, between the F1 hybrid and its homozygous parents, attributable to genes linked with the eight marker loci is 8 times this, or 29.8 ± 71.6% of the F2 mean. Using the data in Table 6, we find that the F2 mean is 119.0 g and 29.8% of this is 35.5 g. This is one-quarter of the difference of 141.5 g between the F1 and the mean of its two parents.

Regression coefficients, for quantitative traits on the number of heterozygous loci per plant, are presented in Table 9. The regression coefficients were significant, at the 1% level, for eight quantitative traits; plt ht, ear lgth, ear diam, cob diam, seed yld/ear, seed yld/plt, ear ht, and stalk diam. Two estimates of the total

Table 8. Mean (± standard error) values for 11 quantitative traits of individuals with various numbers (up to 8) of loci heterozygous

Trait[a]	No. of loci heterozygous							
	1	2	3	4	5	6	7	8
Plt ht	169.1± 6.8	167.6±3.4	173.8±1.6	169.6±1.4	174.7±1.5	177.3±2.1	182.2±3.8	180.8± 9.3
Ear lgth	15.7± 1.1	15.6±0.4	16.6±0.2	16.4±0.2	16.9±0.2	17.2±0.3	17.5±0.3	17.4± 0.6
Ear diam	44.0± 1.0	41.3±0.7	43.1±0.3	43.2±0.3	43.3±0.3	43.9±0.4	44.3±0.6	43.8± 1.8
Seed rows/ear	16.4± 0.4	16.0±0.3	16.5±0.2	16.0±0.2	16.6±0.2	16.7±0.2	16.5±0.6	18.0± 0.6
Cob diam	23.9± 0.4	23.1±0.4	23.8±0.2	23.6±0.1	23.9±0.2	24.4±0.2	24.5±0.3	24.6± 1.0
Seed lgth	11.2± 0.3	11.0±0.2	11.3±0.1	11.3±0.1	11.3±0.1	11.4±0.2	11.1±0.2	11.4± 0.2
Seed wt	20.5± 0.8	18.3±0.7	19.2±0.3	20.2±0.2	19.9±0.3	19.7±0.4	19.9±0.6	18.7± 1.1
Seed yld/ear	113.8±11.9	102.1±6.3	115.4±3.2	117.4±3.2	123.9±3.4	125.4±4.4	131.7±6.6	132.3±15.3
Seed yld/plt	127.3±13.8	102.1±6.3	118.3±3.3	118.3±3.2	125.3±3.4	129.3±4.6	131.7±6.6	132.3±15.3
Ear ht	78.5± 6.6	79.6±2.8	81.1±1.6	78.9±1.4	82.5±1.5	84.8±2.3	88.5±3.8	94.4± 9.2
Stalk diam	24.7± 0.9	24.0±0.5	25.3±0.3	25.2±0.3	25.5±0.3	26.1±0.4	25.9±0.8	25.0± 0.5
No. of individuals	9	35	99	129	104	59	20	5

[a] See text for definitions of traits

Table 9. Mean and standard deviation (SD) of each variable, correlation (r) and regression (b) coefficients of each variable with the number of heterozygous loci per individual, probability (*P*) of a greater regression coefficient on the hypothesis that $\beta \leqq 0$, and the total contribution to heterosis in standard deviations of heterozygosity at all 8 loci estimated by regression (8b/SD) and by the sum (Σ d) of the dominance effects for individual loci

Variable	Mean	SD	r	b	*P*	Heterotic contribution	
						8b/SD	Σ d[a]
No. het. loci	4.23	1.40					
Plt ht	173.16	16.88	0.16	1.99	0.00018	0.944	0.781
Ear lgth	16.67	2.35	0.17	0.29	0.00008	0.998	1.034
Ear diam	43.23	3.56	0.12	0.32	0.00368	0.714	0.614
Seed rows/ear	16.40	1.98	0.09	0.12	0.03070	0.498	0.429
Cob diam	23.85	1.78	0.16	0.20	0.00025	0.921	0.961
Seed lgth	11.27	1.09	0.05	0.04	0.13370	0.297	0.283
Seed wt	19.78	3.06	0.07	0.16	0.05480	0.428	0.362
Seed yld/ear	119.02	35.09	0.18	4.43	0.00006	1.011	0.984
Seed yld/plt	120.98	35.33	0.16	0.45	0.00019	0.940	0.936
Ear ht	81.58	16.46	0.13	1.58	0.00193	0.768	0.676
Stalk diam	25.34	3.18	0.12	0.28	0.00427	0.699	0.862

[a] Obtained from the sum of the mean differences between heterozygotes and homozygotes at all loci given in the last column of Table 4

contribution to heterosis, in F1 plants, of genes linked to the eight marker loci, are presented in Table 9. The first estimate was obtained by using the regression coefficient, b, and the F_2 phenotypic standard deviation, SD, to calculate the standardized difference, 8b/SD, between plants which were heterozygous for all eight loci and plants which were homozygous. The sum of the individual dominance effects, Σd, for all eight loci was used as a second estimate. This estimate was obtained from the last column of Table 4. Both estimates are expressed in F2 standard deviation units.

For traits such as seed yld/ear, the total difference between plants like the F1 hybrid, which were heterozygous for all eight maker loci, and plants like the inbred parents, which were homozygous for all eight loci, is close to one standard deviation. This is about one-quarter of the difference in seed yld/ear between the F1 hybrid and the mean of its parents (see Tables 6 and 9).

Discussion

All enzyme loci were strongly associated with at least one quantitative trait, and all quantitative traits had significant associations with genotypes at particular

enzyme loci in the F2 population derived from the cross between the inbred lines Wf9 and Pa405. Highly significant associations with the number of heterozygous loci per individual were found for 9 of 11 quantitative traits. Significant associations were too frequent to be due to chance.

Soller et al. (1976) derived an equation which can be used to calculate the sample size necessary to detect a given true mean difference between genotypes for any level of significance and power. They felt that a true mean difference between two genotypes of 0.282 standard deviations (SD) would be "a reasonable magnitude of effect to look for". Their calculations show that in samples of 525 individuals, with two equally frequent phenotypes, such a true mean difference would be declared to be significant, at the 5% level, 90% of the time when two-tailed tests are used.

The differences between heterozygote and homozygote means in our experiment were based on samples of 460 plants. Furthermore, we tested the one-tailed hypothesis that the means for heterozygotes are not greater than means for homozygotes. Using Soller et al.'s formula, we calculated that the probability of finding a dominance difference to be significant at the 5% level is: 0.85 when the true difference is 0.25 SD; 0.69 when the true difference is 0.20 SD; and 0.49 when the true difference is 0.15 standard deviations.

The dominance differences, between heterozygote and homozygote means, are given in Table 4. Significant differences ranged in magnitude from just over 0.15 to 0.30 standard deviations. Locus *Got1* had significant dominance differences for plt ht, seed yield, and seed yield related traits such as ear lgth and cob diam. Whether these dominance differences are due to one highly pleiotropic gene or due to several genes with separate functions will require further investigation. *Glu1* dominance differences were restricted to seed yield and the important yield component, ear lgth. The *Glu1* region had the greatest dominance effect with almost a third of the total contribution, by all marker loci, to heterosis.

Segregation distortion (Table 1) was found for the *Glu1* locus, where only 19%, instead of the expected 25%, of plants were of the 22 genotype. The gene or genes which caused the deviation from a 1:2:1 ratio also may have caused the high apparent overdominance of *Glu1* for yield related traits: the infrequent 22 genotype had a seed yld/plt of only 113.9 g compared with 117.2 g and 125.7 g for the 11 and 12 genotypes, respectively.

Large dominance effects are definitely not ubiquitous. Locus *Mdh2* like *Pgd1* is located on the long arm of chromosome 6, but *Mdh2* did not come close to having significant dominance effects on any trait. However, this does not mean that the chromosomal region where *Mdh2* is located is devoid of genes with dominance effects. Soller et al. (1976) have shown that the "apparent heterotic effect" of a maker locus is proportional to $R = 1/(1-2r)^2$, where r is the probability of recombination between the marker locus and a locus with a purely overdominant effect on a quantitative character. This means that if, for example, $r = 0.20$, the proportionality factor would be 2.78. In order to be detected half the time at the 5% level of significance, quantitative trait genes would need to have dominance effects of more than 2.78×0.15 or 0.42 standard deviations.

Our tentative conclusion is that the two parent inbred lines, Wf9 and Pa405, have genomes which differ with respect to only a very few genes, with effects greater than 0.30 standard deviations, per chromosome.

Overdominance for seed yield

The Wf9 and Pa405 inbred lines were selected to produce an F1 hybrid with very high seed yield. Our original expectation was that regions of the genome marked by enzyme loci would exhibit an intermediate degree of dominance for most traits, including seed yield.

Four loci, *Got1, Est4, Glu1,* and *Pgd1,* had significantly higher heterozygote than homozygote means for seed yld/plt and/or PC 1, which is highly correlated with yield related traits and accounted for over 42% of the variation in all standardized trait variables (Table 4). The two homozygote genotypes at each of the four loci did not differ significantly for seed yld/plt or PC 1 (Table 5).

Relative to the midparental value for seed yld/plt, the ratio of the heterozygote effect d to the effect a of the best homozygote were: 0.173 to 0.076 SD for *Got1;* 0.151 to 0.040 SD for *Est 4;* 0.277 to 0.047 SD for *Glu1;* and 0.162 to 0.065 SD for *Pgd1.* Contrary to our original belief, it appears that most of the heterosis observed in the F1 hybrid between Wf9 and Pa405 is attributable to genes associated with short regions of the genome with remarkably overdominant effects on seed yield.

Comparison of the F2 results with those from a mass selected population

Pollak et al. (1984) found a number of genotype-trait associations in two experimental open-pollinated populations of maize. A comparison of their study with ours showed that both studies included seven of the same enzyme loci (*Acp1, Got1, Prx1, Mdh2, Est4, Pgd1, Glu1*) and four of the same quantitative traits (plt ht, ear ht, yld/ear, yld/plt). In their study, 15 of 17 analyses indicated significant ($P \leqq 0.05$) associations between quantitative traits and genotypes at enzyme loci, but the nature of the associations were different between

the two studies. For example, in the 'Hays Golden' populations, *Acp1* was associated with seed yield, maturity and leaf variables, whereas, in the F2 population of Wf9 × Pa405, *Acp1* was associated with ear lgth and cob diam but not individual seed yld/plt. In the F2 population, *Glu1* was strongly associated with ear lgth, seed yld/ear, and seed yld/plt, but *Glu1* was not associated with any morphological trait in the Hays Golden populations.

It is likely that the associations, which were found, depend heavily on the nature of the material studied and possibly even on genotype-environment interactions. Pollak et al. (1984) used open-pollinated populations undergoing mass selection. Any disequilibrium due to selection would tend to be reduced or lost during several generations of random mating. In the F2 of a cross between homozygous lines, extreme gametic phase disequilibrium can exist between closely linked genes and cause associations between enzyme loci and quantitative traits.

The results of this study suggest that quantitative trait-enzyme marker locus studies of sets of three or more different inbred lines, with at least two different alleles at a locus expressed among the inbreds, will permit the detection and transfer of genes with beneficial effects on agronomically important traits. Thus, it may be possible to utilize multiple enzyme marker loci to identify pairs of inbred lines which, when in hybrid combination, exhibit heterosis for yield greater than currently available.

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