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Molecular-Marker-Facilitated Investigations of Quantitative-Trait Loci in Maize. I. Numbers, Genomic Distribution and Types of Gene Action

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Molecular-Marker-Facilitated Investigations of Quantitative-Trait Loci in Maize. I. Numbers, Genomic Distribution and Types of Gene Action

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ABSTRACT

Individual genetic factors which underlie variation in quantitative traits of maize were investigated in each of two F_2 populations by examining the mean trait expressions of genotypic classes at each of 17–20 segregating marker loci. It was demonstrated that the trait expression of marker locus classes could be interpreted in terms of genetic behavior at linked quantitative trait loci (QTLs). For each of 82 traits evaluated, QTLs were detected and located to genomic sites. The numbers of detected factors varied according to trait, with the average trait significantly influenced by almost two-thirds of the marked genomic sites. Most of the detected associations between marker loci and quantitative traits were highly significant, and could have been detected with fewer than the 1800–1900 plants evaluated in each population. The cumulative, simple effects of marker-linked regions of the genome explained between 8 and 40% of the phenotypic variation for a subset of 25 traits evaluated. Single marker loci accounted for between 0.3% and 16% of the phenotypic variation of traits. Individual plant heterozygosity, as measured by marker loci, was significantly associated with variation in many traits. The apparent types of gene action at the QTLs varied both among traits and between loci for given traits, although overdominance appeared frequently, especially for yield-related traits. The prevalence of apparent overdominance may reflect the effects of multiple QTLs within individual marker-linked regions, a situation which would tend to result in overestimation of dominance. Digenic epistasis did not appear to be important in determining the expression of the quantitative traits evaluated. Examination of the effects of marked regions on the expression of pairs of traits suggests that genomic regions vary in the direction and magnitudes of their effects on trait correlations, perhaps providing a means of selecting to dissociate some correlated traits. Marker-facilitated investigations appear to provide a powerful means of examining aspects of the genetic control of quantitative traits. Modifications of the methods employed herein will allow examination of the stability of individual gene effects in varying genetic backgrounds and environments.

MOST important characteristics of agricultural crops are inherited quantitatively. Since the proposal of the multiple-factor hypothesis by both NILSON-EHLE and EAST in 1909, continuous variation has been thought to arise largely from the collective effects of numerous genes, each having a small effect. Because these effects have not generally been resolvable individually, quantitative geneticists have dealt largely with characterization of these factors *en masse*, using biometrical procedures. Many issues in quantitative genetics and evolution are difficult to address without additional empirical information about the genes which underlie continuous variation. The identification and examination of individual quantitative genes should provide information about the organization of genomes and insight into the relative contributions of "major" and "minor" genes to continuous variation. The ability to identify specific quantitative genes would also lead to a more powerful means of investigating epistasis, pleiotropy and the genetic basis of heterosis. As these aspects of quantitative genetics are increasingly understood, new methods might be developed to contribute to current approaches to plant improvement.

Reports of linkage between quantitative trait effects and major genes (RASMUSSON 1933; EVERSON and SCHALLER 1955) followed one of the earliest such reports by SAX (1923). In a converted effort to locate quantitative factors in wheat (*Triticum aestivum* L.), LAW (1967) used in intervarietal chromosome substitution line to study effects associated with four morphological marker loci on chromosome 7*B*. Factors influencing grain weight, grain number, height and tiller number were identified and mapped with respect to the marker loci. Quantitative factors influencing sternopleural bristle number in *Drosophila melanogas-*

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ter were identified on chromosomes *X, 2* and *3* (SPICKETT and THODAY 1966). Five identified genes accounted for 87.5% of the difference in bristle number between a line selected for high bristle number and the wild type. Mapping these quantitative trait genes by use of marker loci has allowed insight into qualitative differences in their expression as well as examination of their interactions. Until recently, comparably sophisticated examination of factors underlying quantitative trait variation in higher plants has not been possible, largely due to the lack of suitable marker loci.

Recent investigations into electrophoretic variability at enzyme-encoding loci have produced an extensive set of mapped marker loci for some species (TANKSLEY and RICK 1980; GOODMAN *et al.* 1980; STUBER and GOODMAN 1983). These marker loci have the advantage of being largely co-dominantly inherited, allowing complete classification of genotypes in an F_2 population. Isozyme loci have been employed in two reports of marker locus associations with quantitative traits in interspecific crosses of tomato (*Lycopersicon* spp.). TANKSLEY, MEDINA-FILHO and RICK (1982) used 12 isozyme loci to locate factors influencing four quantitative characteristics in a backcross population of 400 plants involving *L. esculentum* and *L. pennellii.* Twenty-seven of the 48 possible comparisons between marker loci and quantitative trait expression were significant, with a minimum of five quantitative trait loci (QTLs) detected per trait. WELLER (1983) examined 18 quantitative traits in an interspecific F_2 of tomato (*L. pimpinellifolium* × *L. esculentum*). Four of the ten marker loci employed were isozyme loci. Eighty-three of 180 possible marker-trait comparisons were significant. Complete classification of marker locus genotypes allowed dominance relationships at QTLs to be examined for 68 marker-trait comparisons. Effects ranged from additivity for 35% of the effects to apparent overdominance (16%). Dominance was generally in the direction of the wild-type homozygote.

Maize (*Zea mays* L.) is an excellent species with which to investigate the location and behavior of factors which underlie quantitative trait variation. Many quantitative traits have already been extensively investigated using conventional biometric approaches (HALLAUER and MIRANDA 1981). Numerous qualitative loci are mapped to each of the ten maize chromosomes and elaborately constructed translocation stocks (SHERIDAN 1982) are available for mapping marker loci (allozyme loci or restriction fragment length polymorphisms) for use in locating QTLs. Allozyme genotypes of 406 public inbred lines at 22 loci have been reported (STUBER and GOODMAN 1983) and provide an information base for selecting lines with different marker-locus genotypes. An additional 17 allozyme loci have been resolved, many of which are mapped (J. F. WENDEL, C. W. STUBER, M. M. GOODMAN and M. D. EDWARDS, unpublished data). Many allozyme loci of maize are polymorphic among cultivated inbreds, allowing marker-facilitated investigations to be conducted without resorting to wide crosses.

The purpose of this investigation was to use segregating allozyme loci in two F_2 populations of maize to locate and study QTLs for a number of traits. Primary interests included inferences regarding the numbers and distribution of QTLs as well as specific insights into their gene action and magnitudes of gene effects.

MATERIALS AND METHODS

Experimental procedures: Two F_2 populations of maize were developed as source material for this investigation by self-pollinating the F_1 hybrids CO159 × Tx303 and T232 × CM37. Inbred parents of these hybrids were chosen to maximize both the number of segregating allozyme loci and segregation for agronomic and morphological characteristics in the F_2 populations. The parental inbreds within each pair are divergent primarily in maturity and plant height since they were developed in the southern and northern corn belt regions. However, all are publically available field corn lines which are adapted to North American agricultural practices and have achieved some degree of commercial utilization. Etiolated coleoptile tissue from 5-day-old F_2 seedlings was sampled and used for electrophoretic analyses as detailed by STUBER and GOODMAN (1983) and CARDY *et al.* (1983). This sampling procedure did not damage seedlings, which were transplanted to 8-cm peat pots and nurtured in the greenhouse for approximately 10 days prior to transplanting to a field near Clayton, North Carolina, in late May 1984. Within each population, individuals were uniformly transplanted to a single, rectangular block in the field at 97-cm row spacing and 30-cm plant spacings within rows. This block was divided into four, approximately square, regions to provide an error term for portions of the statistical analysis. The F_2 populations of T232 × CM37 (CMT) and CO159 × Tx303 (COTX) were represented by 1930 and 1776 plants, respectively, and were segregating at 18 and 15 allozyme loci, respectively, plus two morphological loci, each. These marker loci are distributed on eight of the ten maize chromosomes in each population and are within 20 cM of about 40–45% of the genome (Figure 1).

About 40 quantitative characteristics were measured for each plant throughout the season. Measured traits included weights, dimensions and counts of many vegetative and reproductive plant parts as well as flowering dates. These led to the construction of 82 quantitative traits, many of which are composites of several individual measurements (*e.g.*, harvest index = grain weight/plant total weight). A subset of 25 traits was chosen to represent a range of plant parts and agronomic characteristics and is presented in detail in Table 1. Ears, stalks and leaves were harvested uniformly from all F_2 plants after black-layer formation (about 1 September). Ears were weighed, dried at 35° to approximately 15% moisture, then reweighed to determine percent moisture. Leaves and non-leaf vegetative plant parts (stalks, shanks, husks and tassels) were thoroughly dried in a forced-air dryer prior to data collection. The data from the two populations were analyzed with a program written using SAS's Matrix Procedure (SAS Institute Inc. 1982). This

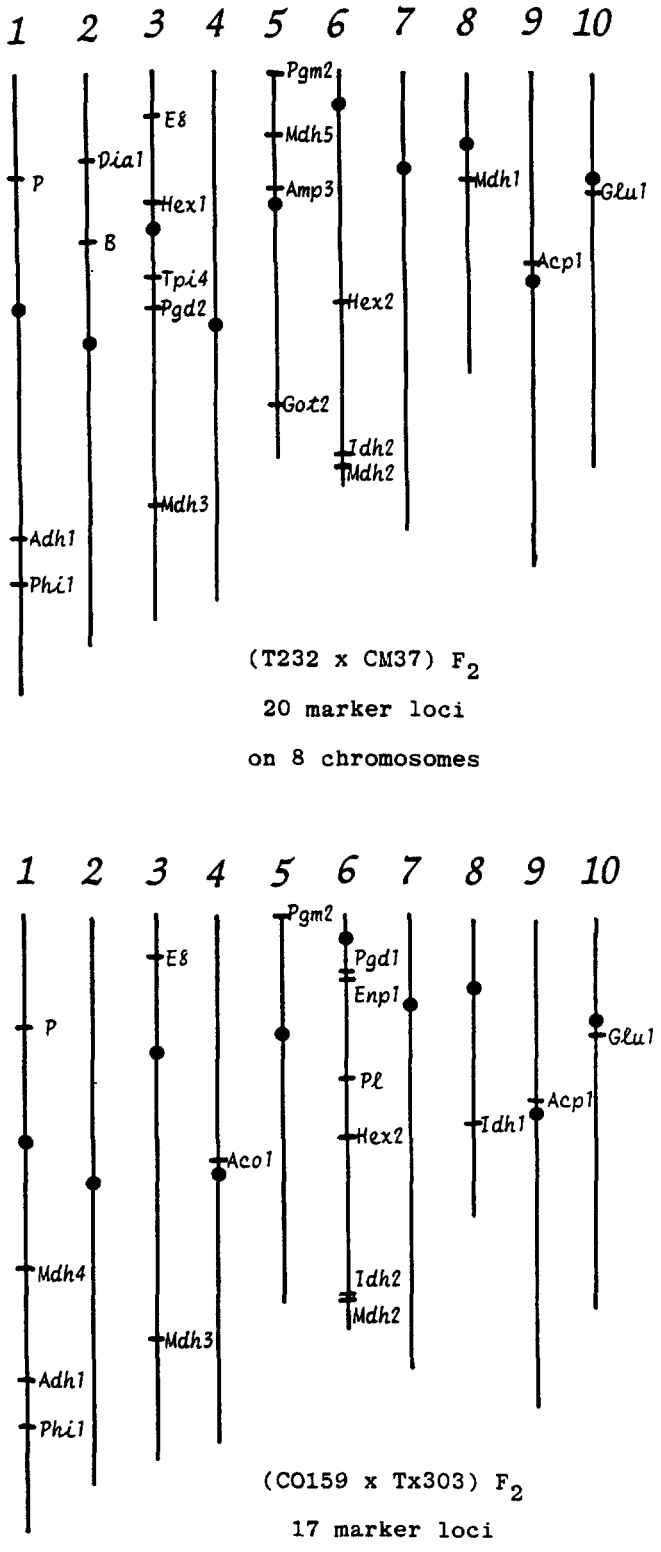


FIGURE 1.—Distributions of the 20 and 17 marker loci segregating in the F_2 populations: T232 × CM37 and CO159 × Tx303, respectively.

program conducted single-factor ANOVA for each pairwise combination of quantitative trait and marker locus. *F*-tests were used to determine if significant variation in trait expression was associated with differences in marker-locus genotypic classes. Significant *F*-values were interpreted to indicate segregation of genotypes at a QTL which is linked to the marker locus. Additive and dominance effects attributable to QTLs were determined from differences between mean trait expressions of marker locus genotypic classes, as discussed below.

Either Bartlett's or Hartley's test for homogeneity of variances was conducted to examine effects of marker-locus associated QTLs on trait variances [see NETER and WASSERMAN (1971), pp. 509–513]. The cumulative prediction of quantitative trait expression from the effects associated with marker loci was determined using SAS's GLM procedure. Genotypes at marker loci were converted into columns of indicator variables upon which trait expressions were regressed to obtain multiple R^2 values. Only main effects at marker loci were modeled in this fashion.

No attempts were made to examine the data using a factorial style of analysis since the number of testable multilocus interactions greatly exceeded the available degrees of freedom, even with the large population sizes employed. Only digenic epistatic interactions were examined. These were tested by determining the mean trait expression of the nine, two-locus genotypic classes for every pair of marker loci that allowed complete classification in the F_2 in each of four geographic regions of the field. These means were then subjected to two-factor analysis of variance, using SAS's GLM procedure with means weighted according to the

TABLE 1

Types of quantitative traits measured in two F_2 maize populations, with descriptions of 25 traits appearing in Table 3

General description of types of traits evaluated:	
Dimensions of:	Leaves, stalk, tassel, ear, shank, kernels
Weights of:	Leaves, stover, ears, kernels
Numbers of:	Leaves, tassel branches, ears, kernel rows, kernels
Ratios of:	Numerous combinations of the above

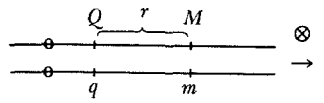
Designations and descriptions of 25 traits presented in detail:

Designation	*Description*
GWT	Plant grain weight in g
E1GWT	Top ear grain weight in g
E2GWT	Second ear grain weight in g
ENO	No. of ears with >1 g grain
ECIR	Top ear circumference at widest point
ELEN	Top ear length
KERDEP	½ of difference between ear and cob diameter
ROWNO	No. of kernel rows on top ear
KERNO	No. of kernels produced by plant
100-SWT	Weight (g) of 100 kernels
PM	Ear % moisture at harvest [(wet wt-dry wt)/dry wt]
EARHT	Height to ear node in cm
EARLEAF	No. of leaf (from ground) subtending ear
LFABOVE	No. of leaves above the ear
LEAFNO	Total no. of leaves
LFDWT	Dry weight of leaves in g
SILK	Days from sowing to top ear silk emergence
HINDEX	Harvest index [(grain wt/(grain + stover wt)]
STKDWT	Stalk dry weight in g
STSWT	Dry weight (g) of 15.25 cm basal stalk segment
TBNO	Total no. of tassel branches
7WK HT	Height (cm) to uppermost leaf tip at 7 weeks
PLT HT	Height (cm) to tip of tassel at maturity
5WK DIA	5-Week stalk basal internode diameter (cm, narrow dim.)
7WK DIA	7-Week stalk basal internode diameter (cm, narrow dim.)

TABLE 2

Basis for interpreting the expression of marker locus genotypes in terms of additive (*a*) and dominance (*d*) effects at a quantitative trait locus linked to the marker locus with recombination frequency, *r*

A. Hypothetical F_1 genotype:



F_1 gametes	*Frequencies*
QM	½ (1 − *r*)
qm	½ (1 − *r*)
Qm	½ *r*
qM	½ *r*

B. Generates F_2 array:

Genotypes	*Frequency*	*Value*
QQMM	¼ $(1-r)^2$	+*a*
QqMM	½ $(r-r^2)$	*d*
qqMM	¼ r^2	−*a*
QQMm	½ $(r-r^2)$	+*a*
QqMm	½ $[r^2+(1-r)^2]$	*d*
qqMm	½ $(r-r^2)$	−*a*
QQmm	¼ r^2	+*a*
Qqmm	½ $(r-r^2)$	*d*
qqmm	¼ $(1-r)^2$	−*a*

C. Marker-locus class means (frequency-adjusted):

Marker class	*Mean expression*
MM	$(1-2r)a + 2r(1-r)d$
Mm	$[(1-r)^2 + r^2]d$
mm	$(1-2r)(-a) + 2r(1-r)d$

D. Expressions to resolve additive and dominance effects:

Additivity: $(MM - mm)/2 = a(1-2r)$

Dominance: $Mm - (MM + mm)/2 = d(1-2r)^2$

E. Dominant/Additive ratio:

$$\frac{Mm - (MM + mm)/2}{(MM - mm)/2} = (1-2r)\,d/a$$

M, *m* and *Q*, *q* are alleles at the marker and quantitative trait loci, respectively.

frequency of individuals in each two-locus class. The (epistatic effects) × (region) interaction was used to test the 4 df digenic epistasis term. Significant epistatic effects were partitioned into additive × additive (A × A), additive × dominant (A × D), D × A, and D × D components.

The effects of marker-locus associated QTLs on trait covariances was examined using an analysis of commonality approach [see KEMPTHORNE (1957), p. 304]. This was equivalent to establishing the partial correlation between two quantitative traits after the effects of a marker locus had been removed by eliminating effects associated with its two columns of indicator variables. The relationship between the original R^2 and the R^2 determined from partial correlation served as a measure of the direction and magnitude of the contribution of each marker-locus region to the overall trait covariance.

Theoretical basis for interpreting QTL-marker locus associations: The use of marker loci to detect individual quantitative trait loci has been examined theoretically (JAYAKAR 1970; MCMILLAN and ROBERTSON 1974) and empirically in a few instances (LAW 1967; SPICKETT and THODAY 1966). The association of marker-loci with quantitative trait expression is most simply interpreted in segregating populations derived from crosses between inbred lines. MATHER and JINKS (1971) have described the basis for interpreting quantitative effects associated with marker loci in backcross (BC_1) progenies, where only two genotypic classes may occur at any locus. The relative powers of backcross and F_2 populations for detecting QTLs were discussed by SOLLER and

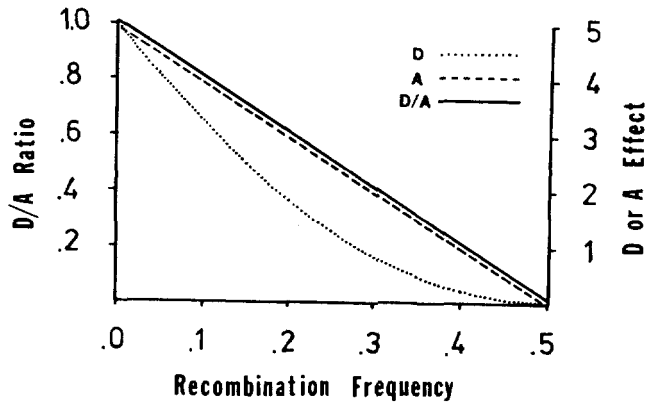


FIGURE 2.—Effects of the recombination frequency between a quantitative trait locus and a marker locus on the dominance (D) or additive (A) effects and the D/A ratio perceived at the marker locus for a hypothetical quantitative locus exhibiting "pure" dominance (D = A = 5 units).

BRODY (1976). The degree of dominance presumed to exist at QTLs affects the relative merits of the population type; however, the F_2 population generally requires fewer offspring for a given power. If all three genotypic classes at marker loci may be identified, the use of F_2 populations also provides information about gene action at identified QTLs (or genomic regions). This aspect has been discussed briefly by SOLLER and BECKMANN (1983).

To examine the relationship between marker-locus genotypes and quantitative trait expression, consider an F_1 derived from two inbreds, for which a chromosome arm is heterozygous at a co-dominant marker locus, designated, *M*/*m*. The F_1 may also be heterozygous at a QTL, *Q*/*q*, which is linked to the marker locus with some recombinant frequency, designated *r* (Table 2A). F_2 progeny, then, will consist of nine genotypic classes with respect to these two loci (Table 2B). The relative frequencies of these genotypes are functions of *r*, and the expressions of these genotypes for a given quantitative trait are assigned based upon the genotype at the QTL. Because genotypes at the QTL cannot be discriminated, their effects must be inferred via association with the genotypes at the linked marker locus.

The sums of frequency times expression, across each marker locus genotype, produce the expected expressions of the three resolvable marker locus genotypes in terms of both *r* and the genotypic effects due to the QTL (Table 2C). Note that these expressions have been adjusted from their relative frequencies of 1/4, 1/2 and 1/4 to unity to allow comparisons among them. The expressions for *MM*, *Mm* and *mm* simplify to the assigned values of QTL genotypes, *a*, *d* and −*a*, respectively, when $r = 0$ (*i.e.*, the marker locus is responsible, itself, for the detected quantitative effects). If $r = 0.5$ (*i.e.*, the marker locus segregates independently of the nearest QTL) all three marker locus genotypic classes have the equivalent value, *d*/2, which is the mean expression of the F_2 population. When $0 < r < 0.5$, the expression of homozygous marker-locus classes are complex functions of both additive and dominance effects. Contrasts involving these class means may be used to develop functions which are simpler expressions of additive or dominance gene effects (Table 2D). The apparent degree of dominance at the QTL can be approximated as the ratio of the "dominance" to "additive" contrasts (Table 2E). Note that this expression is progressively biased in the direction of underestimating the "true" *d*/*a* ratio at the QTL as *r* approaches 0.5 (Figure 2). The magnitude of this bias is fairly small, however, with small values of *r*, when differ-

ences between the expression of marker-locus classes are most likely to be significant.

The above model describes the interpretation of marker locus-QTL associations for the simple case of linkage of one segregating marker with one postulated QTL. Failure to detect a QTL for a trait linked to a particular marker locus does not imply, of course, that there is no QTL in the region. The two parental inbreds may have identical alleles at a linked QTL, which thus escapes detection. Alternatively, the parents may have different alleles at the QTL which have equivalent expressions for the particular trait. The number of genomic regions exhibiting marker-linked effects in an F_2 population must, therefore, represent only a minimum estimate of the number of QTLs effective in determining the expression of the trait, even with sufficient markers to cover the genome completely.

Linkage disequilibria are maximized in an F_2 population, and consequently, rather large genomic regions are represented by marker loci in this generation (HANSON 1959). While this is advantageous for detecting QTLs with a minimum number of markers, it increases the chance that genotypic classes at a marker locus may be reflecting the effects of multiple QTLs which are linked to the marker locus. If this is the case, it can be demonstrated that the effects associated with the marker locus class contrasts are:

$$(MM - mm)/2 = \sum_{i=1}^{n} [a_i(1 - 2r_i)]$$

$$Mm - (MM + mm)/2 = \sum_{i=1}^{n} [d_i(1 - 2r_i)^2]$$

where:

a_i = the additive effect at locus i
d_i = the dominance effect at locus i, and
r_i = the frequency of recombination between locus i and the marker locus, M/m.

Note from the following discussion that multiple QTLs linked to a marker locus may have a dramatic influence on the perceived type of gene action occurring near a QTL. The contrasts are sums of directional additive or dominance effects at multiple contributing QTLs, with each effect diminished to the degree that recombination has dissociated it from the marker locus. Unless the parental inbreds represent divergent extremes for a particular quantitative trait, the additive contrast may underestimate the sum of the individual additive effects due to counterbalancing positive and negative a_i's. Even very divergent material may be unlikely to have unidirectional additive effects at all loci influencing a specific quantitative trait. This interpretation is supported by the effectiveness of reverse selection after 48 generations of divergent selection for oil and protein content in Burr's White maize (DUDLEY 1974). The directionality of dominance effects, d_i's, is not influenced by parental origin of alleles and may be largely unidirectional for some traits regardless of the parental lines involved. According to classical theory (FISHER 1930) selection pressures would tend to promote dominance of favorable alleles. Grain yield of maize is a trait for which the direction of dominance generally might be expected to be positive, due to natural or artificial selection. It seems likely that for some traits, therefore, linkage of multiple QTLs to a marker locus will often result in over-estimation of the dominance/additive ratio of individual loci. SOLLER, BRODY and GENIZI (1979) have investigated the theoretical likelihood of multiple, marker-linked QTLs for a trait. Although they conclude that each marker-linked effect will probably be due to one, or at most, two QTLs, it must be recognized that such inferences are based on extrapolation from assumptions about which we have virtually no empirical information.

RESULTS AND DISCUSSION

Segregation ratios of marker loci: All 17 marker loci scored in the F_2 of (CO159 × Tx303) adequately fit their expected 1:2:1 or 3:1 segregation ratios (data not presented). In the F_2 of the (T232 × CM37) population, however, 12 of the 20 marker loci exhibited significant deviations from their expected segregation ratios. Distorted ratios were observed for most loci on chromosomes *1, 2, 3, 6* and *8*, but none on chromosomes *5, 9* or *10*. Although segregation distortion was widespread in this population, its magnitude was generally small enough to have gone undetected with more routinely employed sample sizes. The average deviation from expected frequencies for genotypic classes at the 12 distorted loci was only 7.1%. Distortion did not consistently favor the allele from either of the two parents, but was always unidirectional for all markers on each chromosome, despite independent assortment of some of the loci involved. The mechanism(s) favoring distortion is not evident but must occur prior to zygote development because ears were fully seeded and kernels exhibited nearly perfect germination. This phenomenon of multilocus and multichromosomal segregation distortion is unprecedented, to our knowledge. Its underlying genetic basis is currently being investigated. The small degree of distortion observed did not affect interpretation of the relationships between quantitative traits and marker loci, however, except for having a minor effect on estimates of the variance due to additive and dominance contrasts.

Numbers of factors influencing the expression of quantitative traits: Eighty-two quantitative traits were examined in each of the populations, COTX and CMT, to establish whether significant differences in trait expression were associated with genotypes at each of the segregating marker loci, as indicated by *F*-tests for each pairwise combination of quantitative trait and marker locus. Significant ($P < 0.05$) associations were found for 830 of 1394 comparisons (60%) in the COTX population and 1079 of 1640 comparisons (66%) in the CMT population. A large proportion of these significant associations were highly significant ($P < 0.001$): 506 of the 830 (61%) and 748 of the 1079 (69%) in the populations COTX and CMT, respectively. Some significant associations were found for every one of the 82 traits in each population, indicating that QTLs could be identified which influenced every trait measured in this investigation. An average of 10.2 and 13.8 marker loci were significantly associated with factors influencing the expression of each trait in the COTX and CMT populations, respec-

TABLE 3

Numbers and magnitudes of effects and predictive powers of marker locus genotypes for 25 quantitative traits in each of two maize populations: (CO159 × Tx303) F_2 and (T232 × CM37) F_2

	(CO159 × Tx303) F_2					(T232 × CM37) F_2				
		Locus *R*-squared[b]		Model[c]	Heterozy[d]		Locus *R*-squared		Model	Heterozy
Trait	No.[a] sign	Min.	Max.	*R*-squared	*R*-squared	No. sign	Min.	Max.	*R*-squared	*R*-squared
GWT	13	0.61	3.50	14.23	3.34	18	0.30	5.08	29.96	5.93
E1GWT	13	0.72	4.86	13.42	4.57	19	0.24	8.62	32.33	8.19
E2GWT	11	0.38	4.15	13.98	0.05	12	0.29	6.55	18.67	0.57
ENO	10	0.35	5.24	12.33	0.19	9	0.23	4.13	9.94	0.38
ECIR	16	0.39	3.47	13.73	3.55	19	0.36	11.30	29.74	2.38
ELEN	10	0.40	4.27	13.78	2.09	19	0.32	4.24	22.07	6.63
KERDEP	17	0.53	2.51	13.54	2.88	15	0.46	9.88	22.55	0.65
ROWNO	12	0.47	3.88	12.44	0.31	17	0.25	5.49	27.33	0.03
KERNO	16	0.37	3.89	14.88	2.14	15	0.29	5.14	24.53	3.65
100-SWT	8	0.36	1.91	7.66	0.66	13	0.38	3.84	14.43	1.07
PM	11	0.25	7.07	19.62	0.02	14	0.33	8.46	34.29	0.10
EARHT	9	0.39	15.08	26.98	0.03	17	0.47	6.81	35.02	0.03
EARLEAF	10	0.37	14.17	25.36	0.01	17	0.45	9.73	26.42	0.07
LFABOVE	10	0.44	3.44	10.35	0.04	9	0.29	2.20	9.38	0.26
LEAFNO	12	0.34	16.27	26.40	0.02	15	0.36	10.41	23.61	0.01
LFDWT	12	0.51	15.22	26.99	0.22	14	0.37	6.27	29.17	2.80
SILK	11	0.39	15.63	36.73	0.81	13	0.36	12.99	39.33	1.66
HINDEX	9	0.50	7.06	16.50	1.55	11	0.23	1.88	9.95	0.89
STKDWT	8	0.54	14.82	25.94	0.05	19	0.42	7.42	31.77	2.86
STSWT	9	0.36	7.04	17.59	0.01	16	0.42	6.89	22.56	2.03
TBNO	11	0.42	4.91	27.16	0.00	14	0.32	9.10	22.45	0.09
7WK HT	14	0.40	2.15	11.88	1.80	15	0.31	5.36	19.53	5.99
PLT HT	7	0.85	13.94	28.08	0.23	14	0.38	9.72	39.81	1.35
5WK DIA	9	0.38	2.08	8.46	1.45	13	0.28	1.56	8.38	2.22
7WK DIA	8	0.35	3.60	12.20	0.63	12	0.34	8.17	21.75	2.12

[a] Number of marker loci showing significant association with quantitative trait expression.
[b] Minimum and maximum percent of the phenotypic variation explained by genotypic classes at marker loci that exhibited significant associations with trait expression.
[c] Percent of the phenotypic variation explained by a regression model including simple effects at all marker loci.
[d] Percent of the phenotypic variation explained by the proportion of plants' marker loci which were heterozygous (of 15 loci which allowed complete classification of genotypes in each population).

tively, although numbers varied somewhat among traits (Table 3). Some of these marker loci are linked to one another in each population (Figure 1), and thus may reflect the effects of common quantitative trait loci. For some traits, then, the actual number of separate QTLs identified will be less than the number of significant associations. Closely linked locus pairs such as *Idh2-Mdh2* and *Pgd1-Enp1* on chromosome *6* are rather certain to reflect the effects of the same underlying factors. Interpretation of associations with more loosely linked pairs is less evident, although they ultimately may be more informative [MATHER and JINKS (1971), pp. 13–17]. The number of significant associations with marker loci are presented in Table 3 for 25 quantitative traits examined in both populations. These range from the low values of seven and nine to high values of 17 and 19 of the 17 and 20 marker loci segregating in the COTX and CMT populations, respectively. Yield and many yield-related traits, such as kernel number, kernel depth and ear length and circumference were affected by factors associated with a large proportion of the marker loci (STUBER, EDWARDS and WENDEL, 1987).

Proportion of phenotypic variation explained by individual marker loci: Marker-locus prediction of trait expression was determined by calculating R^2 values due to regression of trait performances on marker locus genotypes (for single loci). The distribution of R^2 values was dramatically skewed, with far greater frequencies of loci accounting for very small than for large R^2 values (Figure 3). The maximum R^2 for any trait due to a marker locus was 16.3% (Table 3). Small R^2 values may reflect either QTLs having only a small effect, or QTLs having a larger effect but being more loosely linked to the marker locus. Sixty-five and fifty-six percent of the 1230 single-locus regressions examined accounted for less than ¾ of 1% of the phenotypic variation observed in the 82 traits examined in the COTX and CMT populations, respectively. In a number of cases, however, genotypes at a marker locus accounted for an appreciable degree of the total phenotypic variation. Forty-seven of the

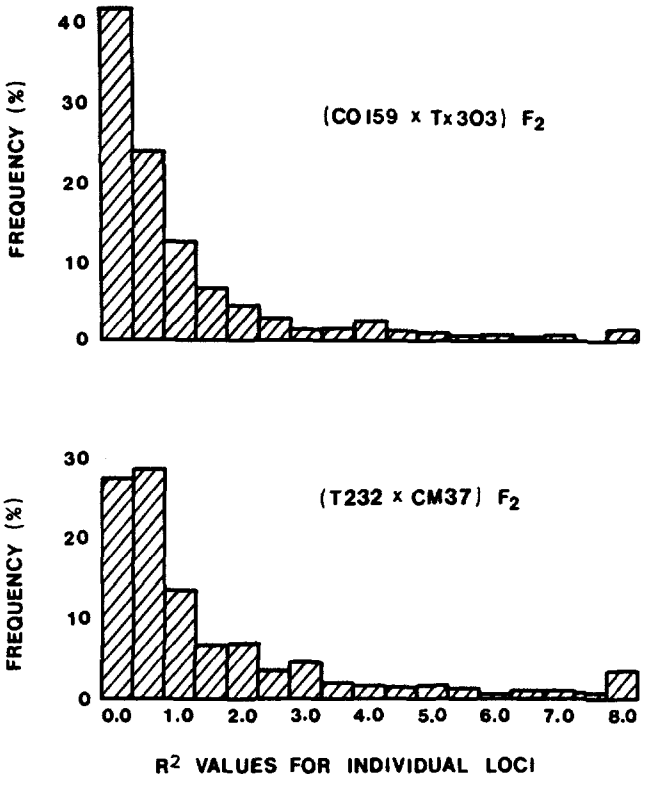


FIGURE 3.—Distributions of R^2 values attributable to main effects of marker loci associated with significant differences in the expression of 82 quantitative traits in the maize populations: (CO159 × Tx303) F_2 and (T232 × CM37) F_2.

1230 single locus regressions (3.8%) in the COTX population and 88 of 1230 (7.2%) in the CMT population exhibited R^2 values greater than 5%. This magnitude of prediction due to a single locus may be better appreciated by considering an example. Genotypes at the *Adh1* locus in the COTX population accounted for only 3.5% of the phenotypic variation in grain yield, yet the difference between grain weight of the two homozygous classes at this locus was greater than 20 g/plant (or 16% of the mean grain yield in the F_2, 127.8 g/plant).

Cumulative proportion of phenotypic variation explained by marker loci: A multilocus R^2 was calculated to determine how much of the total phenotypic variation for each trait could be explained by the cumulative, simple effects of all marker loci. This was done by converting the three genotypes at each locus to two columns of indicator variables, then fitting these indicators sequentially to the individual trait expressions using SAS's GLM Procedure. These models explained a significant ($P < 0.0001$) proportion of phenotypic variation for each of the subset of 25 traits examined in both populations (Table 3). The models accounted for 8–37% of the phenotypic variation in the 25 traits in the COTX population, and 8–40% in the CMT population. Plant height, ear height and days to flower were explained rather well in both populations, having multiple R^2 values ranging from 27 to 40%. Marker locus genotypes in CMT accounted for 30% of the variation in grain weight, but prediction in COTX was poorer, with a multiple R^2 of only 14%. Variation in traits of CMT was generally, but not always, better predicted by marker locus genotypes than that of COTX traits.

Marker locus genotypes of plants cannot predict any variability which is environmental in origin or due to QTLs in unmarked genomic regions. The multilocus R^2 values obtained appear rather large, then, when one considers that they represent effects of only a fraction of the genome and that they are bounded by trait heritabilities. HALLAUER and MIRANDA (1981) summarize numerous estimates of heritability in maize. These range from less than 0.30 for grain weight and kernel depth to between 0.50 and 0.70 for plant height, ear height, kernel row number and days to flower. The "heritability" that is pertinent here could more accurately be termed "the coefficient of repeatibility within an environment," *i.e.*, it includes both genotypic and genotype × environmental variance ($G \times E$) in the numerator. The magnitude of $G \times E$ is not directly addressable with this type of experiment, although $G \times E$ may be eliminated by evaluating the effectiveness of selection within these populations when selection is based upon estimated genotypic values of individuals. Selected progenies may be evaluated in other environments such that the gain from selection reflects genotypic but not $G \times E$ effects. These studies are currently underway.

Effects of heterozygosity on trait expression: The effects of heterozygosity on trait expression were investigated by examining the relationship between percentage of marker loci heterozygous in individual plants and mean trait values. It should be noted that examination of the relationship between heterosis and heterozygosity in artificial populations such as these avoids problems often encountered in natural populations since the allele frequencies here are: $p = q = 0.5$ at each locus (CHAKRABORTY and RYMAN 1983). Fifteen marker loci in each population allowed complete classification of genotypes in each population and were used to estimate heterozygosity levels of plants. Individuals ranged from 0 to 14 loci heterozygous in COTX and from 1 to 15 loci heterozygous in CMT. The regression of each plant's expression for each of 25 traits on its percent heterozygosity was used to determine the proportion of the observed phenotypic variation that could be explained by heterozygosity *per se*, without regard to constitution at specific loci. Variation in 16 and 19 of the 25 traits in the two populations, respectively, was significantly associated with percent heterozygosity (Table 3). The proportion of the phenotypic variance "explained" by percent heterozygosity ranged from 0.2 to 4.6% in COTX and from 0.3 to 8.2% in CMT. Percent het-

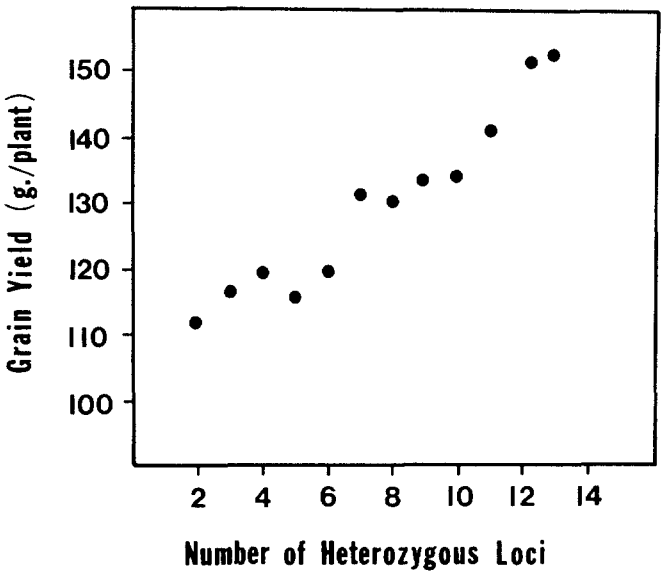


FIGURE 4.—Relationship of mean grain yield with number of heterozygous marker loci in the (CO159 × Tx303) F_2 population.

erozygosity generally accounted for a greater proportion of the variance for grain weight and related characteristics (ear length and circumference, kernel depth and kernel number) than for vegetative traits. Weight of the top ear was the trait most highly associated with heterozygosity in both populations. Heterozygosity exhibited only a fraction of the predictive power for second ear weight that it did for top ear weight and was non-significant in the COTX population. Percentage heterozygosity was not a significant predictor of leaf number, ear leaf node, ear height or tassel branch number in either of the two populations.

The 15 loci that provided information about level of heterozygosity of plants in the population represent only a small portion of the total genome. This fact may contribute to the rather low, albeit significant, prediction of trait expression based upon this measure of heterozygosity. A better estimation of the importance of heterozygosity to trait expression might be obtained by examining the mean level of trait performance observed for plants with a given number of the 15 marker loci in a heterozygous state. Such means should be less affected by error in the estimate of heterozygosity. When examined in this fashion (Figure 4), it is obvious that level of heterozygosity plays a very large role in expression of grain yield, as one would expect.

Association between marker-locus classes and trait stability: Hybrids have often been reported to exhibit less environmental variability than inbred lines (ADAMS and SHANK 1959; LERNER 1961), leading to considerable speculation about the source of heterozygote superiority with respect to stability (ALLARD and BRADSHAW 1964). Trait variances of the three genotypic classes at each marker locus were examined in this investigation to establish whether marker-linked QTLs might affect the stability of trait expression. An initial examination using Hartley's test revealed significant ($P < 0.01$) differences in trait variances between genotypic classes at marker loci in 28 and 35% of the marker locus-quantitative trait comparisons examined in the CMT and COTX populations, respectively. The frequency of such differences in variance was even greater among genotypic classes at marked regions that also affected the mean expression of quantitative traits. Some of these cases may be due to the tendency for means and variances to be correlated, and may thus be considered artifacts of the scale of measurement. [FALCONER (1981), Ch. 17]. Marker loci with nonsignificant effects on the mean expression of a given trait, nevertheless, significantly affected the trait variances in 18 and 21% of the comparisons in COTX and CMT.

Unfortunately, F-tests are notably non-robust as a means of testing for equality of variances, being particularly sensitive to kurtosis in the underlying distributions (BOX 1953; LEVENE 1960). Such tests are, however, quite robust with low kurtosis [BOX (1953), Table 1]. A separate compilation of frequencies of heterogeneous variances was obtained, therefore, using the 21 and 25 traits that exhibited kurtosis values between −0.5 and 0.5 in the COTX and CMT populations, respectively. BARTLETT'S test for homogeneity of variances indicated significant ($P < 0.05$) differences between variances in this subset of traits in 23 and 17% of the examined cases in the two populations, respectively. We conclude, therefore, that marker-linked genomic regions commonly affect trait variances.

Pairwise comparisons of variances among genotypic classes were examined to determine whether heterogeneous variances often resulted from decreased stability (greater phenotypic variance) of homozygous classes when compared to heterozygous classes at individual genomic sites. Converse to this expectation, it was found that homozygous class variances were different from one another 7 and 9% more frequently than were heterozygote vs homozygote comparisons in the COTX and CMT populations, respectively. Among the traits with low kurtosis, for which heterogeneity of variance is most certain, heterozygous classes exhibited variances intermediate between those of the homozygous classes in 65 and 53% of the cases with heterogeneous variances in the COTX and CMT populations, respectively. When heterozygote variances were not intermediate, they were greater than homozygote variances almost as frequently as they were smaller in magnitude; average values for heterozygous classes (across traits and marker loci) were −0.5 and +0.7% of the mid-parent variances of homozygous classes in the COTX and CMT populations, respectively. For grain yield and related traits,

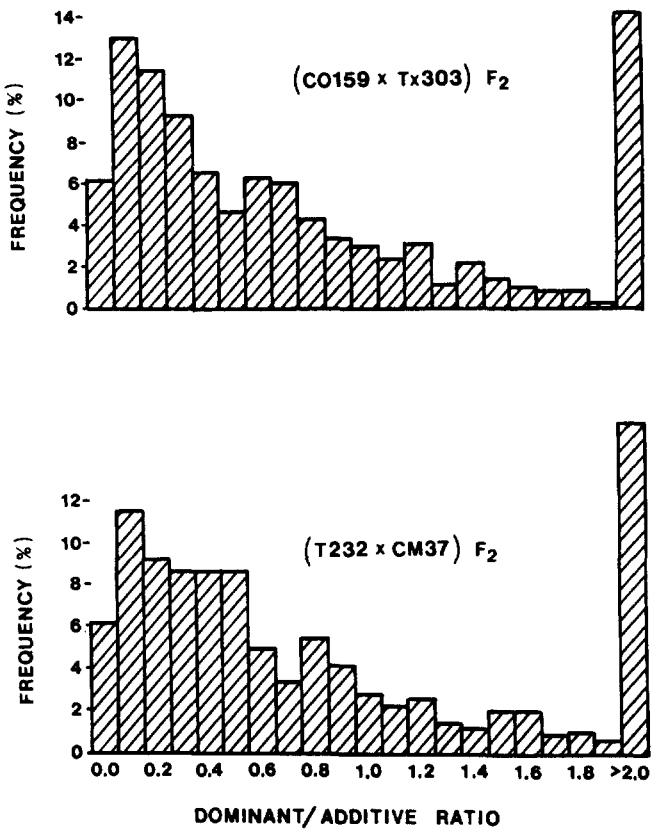


FIGURE 5.—Distributions of dominant/additive effect ratios for marker-locus-associated factors influencing 82 quantitative traits in the maize populations: (CO159 × Tx303) F_2 and (T232 × CM37) F_2.

the magnitudes of heterozygote class variances were, on average, 1–3% below corresponding mid-parent values. It appears that genetic homeostasis in these populations is influenced more by effects of alleles in the marked regions than by heterozygosity, *per se*, at the marked regions. This suggests that the greater yield stability of hybrids when compared to inbreds may not arise from increased stability of heterozygotes at any locus. Instead, as in the argument for apparent overdominance effects arising from complementation of partially dominant genes (JONES 1917), their stability may result from repulsion-phase linkage of many genes influencing stability, with heterozygotes at a given locus generally intermediate but often more stable than the mid-parent.

Types of gene action: The relative importance of dominant and additive gene action was estimated by calculating the ratio of dominant to additive effects (d/a ratio) for those marker loci which were significantly associated with variation in trait expression. Observed d/a ratios varied from 0.0 to over 2.0 in each of the populations (Figure 5). Approximately 50% of these cases in each population exhibited d/a ratios less than 0.55, which might be interpreted as additive or only partially dominant in expression. Another 25% might be considered partially dominant or dominant, with d/a ratios falling between 0.55 and 1.25. A large proportion (25%) of the total cases exhibited d/a ratios exceeding 1.25, suggesting apparent overdominant gene action for these regions of the genome. These apparently overdominant factors may not represent true overdominance, because apparent overdominance may also arise from linkages of more than one QTL, each exhibiting only partial dominance (SVED 1972). If one assumes that true overdominance is very rare or nonexistent, then the approximately 25% apparent overdominance observed in these studies would suggest that linkage of multiple factors influencing a single trait is fairly common and alleles at these loci may often be linked in repulsion. This interpretation for apparent overdominance originated with JONES (1917) and was invoked by MOLL, LINDSEY and ROBINSON (1964) as an explanation for the results obtained in their classic biometric experiment.

Individual traits varied somewhat in the types of gene action commonly observed at marked genomic regions. Grain yield and yield components more commonly showed dominant and overdominant effects than did many vegetative characteristics (data not presented). Even for a given trait, however, different genomic regions often exhibited very different types of gene action. Ear height and stalk weight are examples of traits which exhibited gene action ranging from additivity to apparent overdominance, and for which the direction of dominance varied from region to region.

RUSSELL and EBERHART (1970) examined the epistatic interactions between three morphological loci within a series of crosses involving isogenic sublines of the maize inbred B14. Epistasis was important, accounting for an average of 41% of the variation among genotypes in the nine quantitative traits which were examined. In this investigation, digenic epistasis between pairs of marker-linked genomic regions was investigated as a potential source of genetic control for 15 of the 82 traits evaluated. The traits considered included: grain weight, ear height, plant height, kernel number, kernel depth, kernel row number, leaf number, spike length, and tassel branch number, and were selected to represent a fairly wide range of plant characteristics. For each trait, a two factor ANOVA was conducted with each of 182 pairs of marker loci, producing a total of 2730 ANOVAS per population. Seventy-nine and 80 of these ANOVAS indicated a significant epistatic term in the CMT and COTX populations, respectively, a frequency of just under 3% (data not presented). This low frequency of significance falls well within the range which might be attributed to chance alone. In addition, there was little relationship between the magnitudes or types of gene action for main effects and corresponding as-

pects of those epistatic effects which appeared to be significant (data not presented). There appears to be little evidence, therefore, that digenic epistasis is an important source of variation for the range of traits considered in this investigation. This method should have been quite powerful in detecting interactions between the effects of marked genomic regions, if the marker loci are closely linked to the quantitative trait factors. As for the detection of main effects, the power of this approach diminishes as the distance (recombinant fraction) between the marker locus and the QTL increases. Higher-order epistasis may be important for these marker-linked regions. However, the frequency of individuals within multilocus genotypic classes in this experiment would not have been great enough for an adequate test of higher-order epistasis without arbitrarily grouping genotypic classes. This marker-facilitated method estimates epistasis more directly than is possible by partitioning variance components from mating designs. Nevertheless, as has been reported in maize populations based upon more conventional biometric approaches (STUBER, WILLIAMS and MOLL 1973), this investigation would also suggest that simple epistatic interactions do not contribute greatly to the expression of many of the morphological characteristics of maize which are important in agriculture.

Effects of marker-locus associated factors on trait covariances: Marker loci in this investigation were also used to examine the relationships between pairs of traits. Because the 17 and 20 loci segregating in the two populations had such a high frequency of association with quantitative traits, individual marker loci were often associated with variation in many traits (Table 4). The most notable example of multiple-trait association was the case of *Idh1* on chromosome 8 in the COTX population. The expressions of 78 of the 82 quantitative traits examined were influenced by genotypes at this locus. The traits affected to the greatest degree were plant height and ear height, for which the differences between homozygous classes at *Idh1* were greater than one phenotypic standard deviation. Despite this large "single-locus" effect, the distributions of these traits in the population appeared continuous and approximately normal (data not presented). When distributions of the three genotypic classes were examined individually, the differences in their distributions were evident. Because the mean difference in plant height between homozygous classes was almost 26 cm, it is likely that segregation at this locus would appear qualitative in more uniform genetic backgrounds. A qualitative dwarf, compact (*ct*), on chromosome *8* has been reported in maize (NELSON and OHLROGGE 1957). Because the location of *Idh1* relative to *ct* has not yet been established, it is not evident whether the effects detected here may result from effects of previously unreported alleles at the *ct* locus. The marker loci with effects on the smallest number of traits in COTX were *Mdh2* on 6L with 26 of 82 associations significant and *Est8* on 3S with 23 significant associations. Corresponding ranges in CMT were from maxima of 69 of 82 associations significant for *Pgd2* on 3L and *Dia1* on 2S to minima of 35 and 32 for *Got2* on 5L and *Est8* on 3S, respectively.

The marker loci in these F_2 populations probably reflect effects of rather large sections of the chromosome around them, because there was only one opportunity for recombination in each of the gametes from the F_1. It seems likely, therefore, that associations of marker loci with many traits would often be due to groups of linked factors, some of which influence one trait and some of which influence another. It should be possible to resolve cases arising from loose linkage from those due to very tight linkage or pleiotropy by examining changes in the relationships between marker locus genotypes and the expressions

TABLE 4

Numbers of quantitative traits significantly affected by factors associated with marker-locus genotypic classes[a]

Chromosome[b]	Marker locus	(CO159 × Tx303) F_2		(T232 × CM37) F_2	
		Number[c]	Percent	Number	Percent
1	*P*[d]	49	59.8	58	70.7
	Mdh4	66	80.5		
	Adh1	62	75.6	63	76.8
	Phi1	63	76.8	63	76.8
2	*Dia1*			69	84.1
	B			55	67.1
3	*Est8*	23	28.0	32	39.0
	Hex1[d]			51	62.2
	Tpi4[d]			41	50.0
	Pgd2			69	84.2
	Mdh3	50	61.0	52	63.4
4	*Aco1*	54	65.9		
5	*Pgm2*	44	53.7	69	84.1
	Mdh5[d]			58	70.7
	Amp3			73	89.0
	Got2			35	42.7
6	*Pgd1*	36	43.9		
	Enp1	32	39.0		
	P1[d]	41	50.0		
	Hex2	51	62.2	47	57.3
	Idh2	31	37.8	38	46.3
	Mdh2	26	31.7	36	43.9
8	*Mdh1*			63	76.8
	Idh1	78	95.1		
9	*Acp1*	63	76.8	62	75.6
10	*Glu1*	61	74.3	45	54.9

[a] Marker loci are arranged in chromosomal order.
[b] See Figure 1 for approximate chromosomal positions.
[c] Number affected of 82 traits measured.
[d] Locus which was scored in 3:1 ratio, all others 1:2:1.

TABLE 5

Percent change in the relationship (R^2) between correlated trait pairs due to adjustment for marker locus associated effects in two maize populations: (CO159 × Tx303) F_2 (COTX) and (T232 × CM37) F_2 (CMT)

	Grain weight—ear height		Grain weight—ear number		Grain weight—percent moisture	
Marker locus	COTX	CMT	COTX	CMT	COTX	CMT
P	−2.21	−4.43	−6.51	−0.07	−2.79	−4.76
Mdh4	−1.67		−1.88		−15.36	
Adh1	+0.05	+7.87	−0.33	−2.13	−17.16	−0.89
Phi1	−4.31	+7.51	−6.67	−2.58	−17.68	−2.23
Dia1		−3.37		−9.54		−13.96
B		−4.12		−0.99		−10.52
Est8	+0.59	−1.02	+0.00	+0.79	+0.30	−0.60
Hex1		−4.91		−7.12		−2.13
Tpi4		−0.58		−0.83		−0.07
Pgd2		−4.23		−2.91		−3.88
Mdh3	−2.38	−0.51	−1.37	−1.09	−19.71	−0.15
Aco1	−1.73		+1.67		−2.90	
Pgm2	−0.75	−3.65	−0.56	+7.84	−2.46	−11.98
Mdh5		−5.83		−8.44		−17.74
Amp3		−3.29		+8.17		−13.72
Got2		+0.54		+0.86		−0.50
Pgd1	−0.40		+0.16		−0.29	
Enp1	−1.02		+0.43		−0.78	
P1	−4.81		−5.82		−1.36	
Hex2	+1.34	−0.41	−2.04	−0.92	−3.58	+1.30
Idh2	−0.48	−0.51	−0.65	−0.23	−3.63	−0.57
Mdh2	−0.43	−0.72	−0.73	−0.43	−4.13	−1.02
Mdh1		−7.64		+0.15		−15.35
Idh1	−7.36			−12.71		−32.52
Acp1	−0.96	−2.95	−0.63	+1.23	−3.07	−4.29
Glu1	+1.05	+0.70	−0.06	+0.78	−2.39	−0.36
Overall r =	−0.38	−0.56	+0.32	+0.48	+0.17	+0.41
R^2 =	0.144	0.314	0.102	0.16	0.029	0.168

of associated traits in subsequent generations with greater opportunity for recombination.

Whatever the cause of these marker-linked multiple trait effects, the marker loci allow separate examination of the relationship between traits at each marker-linked genomic site. The phenotypic correlation between two traits, thus, may be partitioned into components attributable to individual marker-linked regions of the genome. The effects of marker-linked genetic factors on the relationship between two traits may be indicated by examining changes in the coefficient of determination between the traits which arise from removal of effects due to a marker locus. Individual regions were found to differ in the direction and magnitude of their "contribution" to the overall relationship between some correlated traits examined (Table 5). Tests of significance for these changes in R^2 have not been developed. Loci evidently vary, however, in their effects on the relationship between grain yield and ear height, from those whose removal increases the relationship nearly 8% in CMT to those which accounted for up to 7 or 8% of the observed relationship in both populations. Similar variation in effects was observed for the relationship between grain weight and ear number. Few regions of the genome appeared to act in opposition to the overall direction of the correlation between grain weight and grain moisture at harvest. Even for this correlation, however, regions appeared to contribute to widely varying degrees.

CONCLUSIONS

Segregating isozyme loci in F_2 populations appeared to provide an effective means of identifying genomic regions influencing a wide array of phenotypic characteristics of maize. Every measured characteristic was influenced by segregation at some identifiable genomic regions. The number of plants examined was sufficient to detect factors contributing as little as 0.3% of the phenotypic variation in quantitative traits. Substantially fewer plants would be required to detect the larger effects which were observed for many traits.

The true magnitude of the detected factors cannot be determined without additional information because detected effects are influenced by recombination between marker loci and QTLs. Random-mating

of individuals in these F_2 populations will produce populations with gene frequencies and inbreeding coefficients identical to the F_2, but having increased frequencies of gametes for which recombination had occurred between marker loci and quantitative trait loci. Differences between the quantitative trait expression of marker locus classes in an F_2 and a randomly mated F_2 population may thus be used to estimate the true recombination frequency between the loci and, therefore, also the true magnitudes of gene effects. The number of detected quantitative factors affecting many of the traits in this investigation was great enough to over-account for the total phenotypic variance if all factors were accounting for as much variance as was the largest detected factor. It appears, therefore, that the detected quantitative trait loci differ considerably in the magnitudes of their effects.

QTLs appear to be distributed throughout the genome, with some regions appearing to affect a greater number of traits than others. All marked regions affected at least some traits. Some specific marked regions affected almost all of the traits which were measured; however, it was not possible to determine if these effects were due to several different linked genes or representative of pleiotropy. Further investigations of a similar nature may rule out pleiotropy in some instances but will not be able to conclusively prove its existence at these sites. Whatever the cause of multiple trait effects of these marked regions, these investigations may provide information useful in dissociating trait relationships in these populations.

A wide range of apparent types of gene action was evident at individual regions affecting many quantitative traits. Dominant and overdominant effects were perceived for many traits at a large number of regions. Because the marker-facilitated method tends to underestimate the importance of dominance for marked regions having but one QTL, we might infer that many marked regions may be reflecting the effects of multiple QTLs (in which case this method may result in overestimation of the relative dominance at individual loci). Epistasis (at least, digenic epistasis) does not appear to be important in determining expression of the traits examined.

The 8–40% of the phenotypic variation in quantitative traits that could be explained by cumulative effects of the marked genomic regions seems surprisingly large when one considers the small portion of the total genome which was well-marked in these investigations. The parental inbreds involved in both of these F_2 populations were selected, in part, because they were morphologically divergent; it is not clear, therefore, how representative the observed results are of those that might be obtained using inbreds more similar in appearance. It appears that morphological divergence was not paramount to the success obtained, because the effects of individual regions were occasionally in the opposite direction of parental differences, even in traits for which the parents were the most divergent.

The methods employed here were effective in identifying and locating factors influencing the expression of quantitative traits. Specific inferences are limited, however, to the populations examined and the single environment in which measurements were collected. Further investigations will be required to establish the constancy of the detected effects in other environments and in other genetic backgrounds. Some of these issues may be resolved by subsequent evaluations of material from these investigations in other environments.

Other experimental designs may be devised to optimize the usefulness of marker loci for determining the stability of QTLs in varying environments and genetic backgrounds. For example, the derivation of numerous inbred lines from an F_2 population (via single-seed-descent), would provide reproducible genetic entities that might be analyzed to determine genotypes at numerous marker loci. These lines may be evaluated *per se* or crossed to a number of testers to produce hybrid progeny for quantitative trait evaluation. This would allow both replication in numerous environments and determination of quantitative traits on a plot mean basis (which would minimize environmental sources of variation). In addition, such an approach would reduce the amount of genetic characterization necessary relative to quantitative characterization. In this respect it would be well tailored to the use of restriction fragment length polymorphisms (RFLPs) as markers. RFLPs potentially offer much better genomic coverage than isozymes (HELENTJARIS *et al.* 1985) but currently require both greater expense and effort for genetic characterization.

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