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Molecular-marker-facilitated investigations of quantitative trait loci in maize

4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers

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Summary. Restriction fragment length polymorphisms have become powerful tools for genetic investigations in plant species. They allow a much greater degree of genome saturation with neutral markers than has been possible with isozymes or morphological loci. A previous investigation employed isozymes as genetic markers to infer the location of genetic factors influencing the expression of quantitative traits in the maize population: (CO159 × Tx303)F_2. This investigation was conducted to examine the inferences that might be derived using a highly saturated map of RFLP markers and isozymes to detect quantitative trait loci (QTLs) in the same maize F_2 population. Marker loci that were associated with QTL effects in this investigation generally corresponded well with previous information where such comparisons were possible. Additionally, a number of previously unmarked genomic regions were found to contain factors with large effects on some plant traits. Availability of numerous marker loci in some genomic regions allowed: more accurate localization of QTLs, resolution of linkage between QTLs affecting the same traits, and determination that some chromsome regions previously found to affect a number of traits are likely to be due to linkage of QTLs affecting different traits. Many of the factors that affected plant height quantitatively in this investigation were found to map to regions also including known sites of major genes influencing plant height. Although the data are not conclusive, they suggest that some of the identified QTLs may be allelic to known major genes affecting plant height.

Key words: Restriction fragment length polymorphisms (RFLPs) – Isozymes – Quantitative trait loci (QTLs) – Mapping – *Zea mays* L.

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Introduction

A number of recent investigations have utilized molecular marker loci to examine the inheritance of quantitative traits in maize (*Zea mays* L.) and tomato (*Lycopersicon esculentum* L.) (Tanksley et al. 1982; Kahler 1985; Edwards et al. 1987; Stuber et al. 1987; Nienhuis et al. 1987; Osborn et al. 1987; Paterson et al. 1988; Weller et al. 1988; Tanksley and Hewitt 1988). These studies have generally been successful in identifying some marker-linked chromosome regions that affect a wide range of plant characteristics. Investigations involving codominant markers segregating in F_2 populations have, furthermore, allowed insight into the apparent types of gene action existing at postulated quantitative trait loci (QTLs) in the marker-linked genomic regions (Edwards et al. 1987; Stuber et al. 1987). Many additional questions have been generated by these investigations about the distribution and behavior of QTLs in plant genomes. Some of these questions may, in principle, be addressed by saturating the genome with closely spaced, segregating marker loci. Restriction fragment length polymorphisms (RFLPs) currently provide this potential in some plant species. Helentjaris et al. (personal communication) have developed RFLP probes detecting more than 500 polymorphic loci in maize. Additional maize probes have been developed by other researchers (Burr et al. 1988; D. Grant, Pioneer HiBred, Int., personal communication; Coe et al. 1990). A large number of RFLPs has also been reported in tomato (Bernatzky and Tanksley 1986; Helentjaris et al. 1986; Tanksley and Hewitt 1988). RFLP techniques have the potential of rapidly developing a fairly complete map of linkage groups in a number of other species for which linkage information is currently either very limited or nonexistent.

In this investigation we used 98 RFLP probes and 16 isozyme loci to examine associations between marker loci and quantitative trait expression in an F_2 population of maize that had been previously examined using only isozyme loci as genomic markers. This approach was taken to determine what additional inferences about QTLs might be allowed by more complete coverage of the genome with markers.

Materials and methods

A total of 187 plants from the F_2 population of the cross CO159 × Tx303 was utilized for this investigation. The parental inbreds (Fig. 1) have been described previously (Edwards et al. 1987). Coleoptile tissue was sampled from laboratory-germinated seedlings and preserved at −70 °C for subsequent isozyme characterization, according to previously described procedures (Cardy et al. 1983; Stuber et al. 1988). Seedlings were subsequently transplanted to the field at Clayton/NC in the summer of 1985, at 97-cm row spacings and 30-cm plant spacing within rows. Approximately 3 weeks after the mean flowering data of the F_2 population, leaf samples were collected from the plants in the field for extraction of DNA and subsequent RFLP characterization. One-half of the second leaf above the top ear was sampled from each plant. Leaf samples were rolled and placed in 100-ml test tubes, which were capped and stored on ice until they could be taken to the lab and stored in a −70 °C freezer. At a later date the leaf samples were lyophilized and sent to the lab of T. Helentjaris for RFLP characterization, as previously described (Helentjaris et al. 1986).

One hundred fourteen marker loci were employed in this investigation. Of these, 16 were isozyme loci and the remainder were RFLPs. Some of the probes for the RFLPs were cloned sequences of characterized genes. Most, however, were either random cDNA probes or genomic DNA fragments prepared from *Zea mays*. Table 1 indicates the locus numbers (in chromosomal order) by which these marker loci will be referred herein and simple estimates of recombination frequencies between adjacent loci. Genotypes were not established for every one of the 187 F_2 individuals at each of the 114 marker loci for a variety of reasons: some of which were strategic and some, unavoidable. All ten maize chromsomes were marked with loci, ranging from a minimum of six markers on chromsome 10 to a maximum of 19 on chromsomes 1 and 3. Linkage relationships among marker loci were determined using maximum likelihood algorithms with the assistance of the Pascal program LINKAGE-1 (Suiter et al. 1983). Figure 2 indicates the approximate chromosomal positions of the markers, with consideration given to linkage estimates that span an intervening locus (not presented).

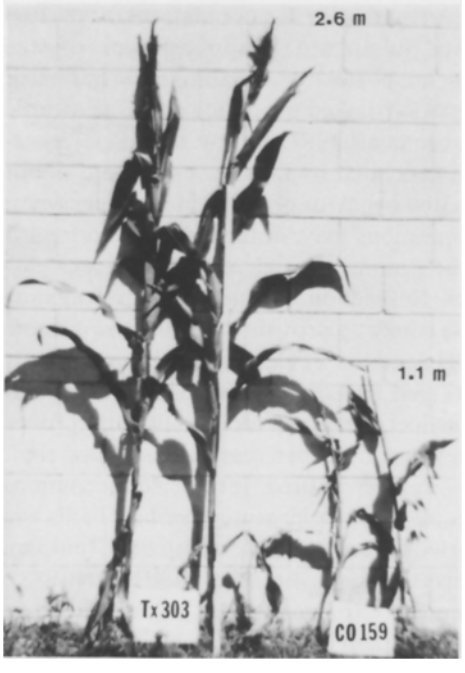

Fig. 1. Phenotypes of the parental inbreds Tx303 and CO159 when grown at Clayton/NC in 1985

Twenty-two quantitative characteristics of the F_2 plants were measured, from which 30 quantitative "traits" were derived. Some traits were direct measurements and others were functions of two or more independent measurements. Table 2 lists the 18 quantitative traits for which data are presented in detail below, as well as a brief description of each trait. The traits were selected to represent a range of plant characteristics and to include traits of agronomic importance.

Analysis of variance was employed to test the significance of effects of marker-linked genomic regions on the quantitative traits, and significant associations were interpreted in terms of underlying gene action, as in a previous investigation (Edwards et al. 1987).

Results and discussion

Analyses of variance revealed that 15.2% of the 3,420 marker locus quantitative trait comparisons were significant at the 5% probability level, 6.2% of the comparisons were significant at the 1% level, and 3.3% were significant at the 0.1% level.

Although the significance levels used are appropriate for individual marker loci, when considering the entire genome the probability of proclaiming a false positive (i.e., committing a Type I error) is much greater than for a single locus. For example, if the 5% probability level is used for individual loci, there is a greater than 99% probability that at least one of the 114 marker locus-quantitative trait comparisons for an individual trait will be falsely judged to be significant. Even at the 0.1% probability level, the overall probability of at least one false positve is about 11%. It should be stressed, however, that placing stringent controls on the Type I error greatly increases the probability of not accepting a real difference (i.e., committing a Type II error). We chose, therefore, the 5% probability level as an appropriate level for judging significance of marker locus-quantitative trait associations in order to adequately control Type II errors.

Isozyme and RFLP markers produced similar frequencies of significant associations with quantitative traits: significant associations (at the 5% level) were detected for 13.8% of 480 comparisons involving isozyme markers and 15.4% of 2,940 comparisons involving RFLPs. Although these frequencies are much lower than

Table 1. Locus numbers by which they are referred in the text and simple estimates of recombinant frequencies for adjacent loci determined by the maximum likelihood method. *Asterisks* (*) indicate nonestimable distances. (Further locus descriptions and map information are available from the authors)

Chromosome 1
1 – 0.04 – *2* – 0.10 – *3* – 0.45 – *4* – 0.23 – *5* – 0.12 – *6* – 0.32 – *7* – 0.15 – *8* – 0.13 – *9* – 0.07 – *10* – 0.26 – *11* – 0.03 – *12* – 0.08 – *13* – 0.04 – *14* – 0.07 – *15* – 0.06 – *16* – 0.09 – *17* – 0.13 – *18* – 0.19 – *19*

Chromosome 2
20 – 0.22 – *21* – 0.25 – *22* – 0.22 – *23* – 0.09 – *24* – 0.22 – *25* – 0.12 – *26* – 0.15 – *27* – * – *28* – 0.05 – *29*

Chromosome 3
30 – 0.15 – *31* – 0.21 – *32* – 0.10 – *33* – 0.19 – *34* – 0.10 – *35* – 0.03 – *36* – 0.10 – *37* – 0.05 – *38* – * – *39* – 0.10 – *40* – 0.12 – *41* – 0.09 – *42* – 0.13 – *43* – 0.07 – *44* – 0.08 – *45* – 0.10 – *46* – 0.14 – *46* – 0.14 – *48*

Chromosome 4
49 – 0.18 – *50* – 0.26 – *51* – 0.21 – *53* – 0.24 – *54* – 0.11 – *55* – 0.13 – *56* – 0.03 – *57*

Chromosome 5
58 – 0.14 – *59* – 0.22 – *60* – 0.13 – *61* – 0.04 – *62* – 0.20 – *63* – 0.21 – *64* – 0.45 – *65*

Chromosome 6
66 – 0.07 – *67* – 0.11 – *68* – 0.03 – *69* – 0.26 – *70* – 0.19 – *71* – 0.12 – *72* – 0.11 – *73* – 0.35 – *74* – 0.05 – *75* – 0.04 – *76*

Chromosome 7
77 – 0.16 – *78* – 0.11 – *79* – 0.20 – *80* – 0.09 – *81* – 0.08 – *82* – 0.31 – *83* – 0.23 – *84*

Chromosome 8
85 – 0.08 – *86* – 0.09 – *87* – 0.13 – *88* – 0.05 – *89* – 0.23 – *90* – 0.04 – *91* – 0.14 – *92* – 0.29 – *93* – 0.11 – *94* – 0.05 – *95* – 0.05 – *96* – 0.13 – *97* – 0.06 – *98* – 0.14 – *99* – 0.25 – *100*

Chromosome 9
101 – 0.18 – *102* – 0.23 – *103* – 0.08 – *104* – 0.04 – *105* – 0.32 – *106* – 0.14 – *107* – 0.47 – *108*

Chromosome 10
109 – 0.20 – *110* – 0.06 – *111* – 0.06 – *112* – 0.09 – *113* – 0.26 – *114*

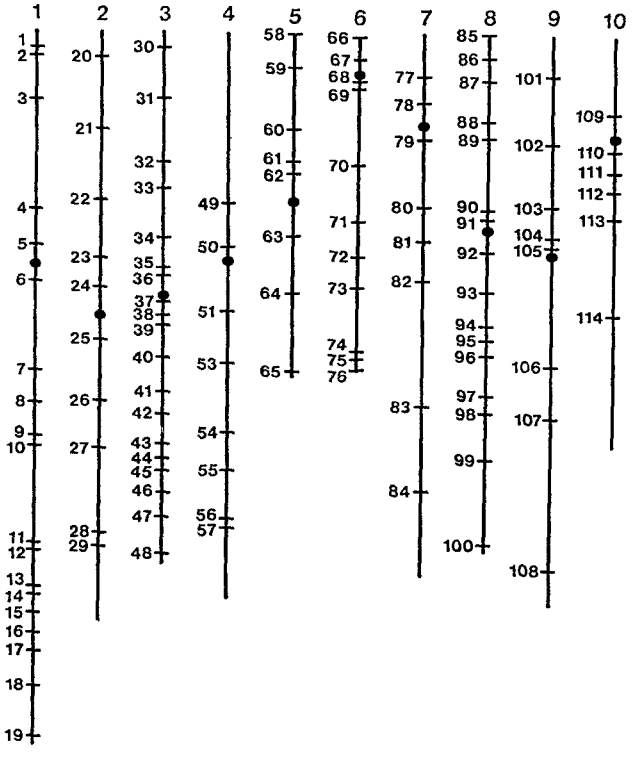

Fig. 2. Approximate centromere locations and chromosomal distribution of marker loci scored in the (CO159 × Tx303) F_2 population. See Table 1 for locus descriptions

the proportion of significant comparisons (approximately 60%) that were reported based upon data collected the previous year when isozymes alone were employed as markers (Edwards et al. 1987; Stuber et al. 1987), this investigation involved about 10% as many plants as were previously evaluated. The reduced plant numbers were necessary because of the increased expense and effort involved in characterization of genotypes at RFLP sites. The necessary methodologies for RFLP characterization are being improved, and somewhat larger investigations are now more feasible. The small size of the population evaluated here results in quite large confidence intervals on estimates of effects associated with marker loci. Any particular association, therefore, may be considerably overestimated or underestimated. Overall, however, the frequencies of significant comparisons at any particular probability of Type 1 error are well above levels that might be attributable to chance alone.

Comparisons were made between the results obtained here and those from a previous study to appreciate the value and limitations of this smaller data set in another year. The ranking of quantitative trait means for genotypic classes at the isozyme loci involved in this investigation were compared with the ranking of means for the same marker locus classes from the 1,776 plants evaluated in the previous study. Such comparisons were made for a subset of eight traits, which were selected because

Table 2. Descriptions of the 18 quantitative traits in the population (CO159 × Tx303) F_2 for which data are presented in detail in the text

Grain weight	Weight (g) per plant of all shelled grain dried to uniform moisture
Ear number	Number of ears per plant with at least 1 g of grain
Ear length	Length (cm) from the butt to the tip of the uppermost ear
Kernel depth	One-half of the difference between the unshelled ear diameter and the cob diameter at mid-ear
Row number	Number of rows of kernels on the uppermost ear
100-kernel weight	Weight (g) of 100 kernels from the uppermost ear dried to uniform moisture
Kernel number	Total number of kernels per plant (calculated from total grain weight and 100-kernel weight)
Percent cob diameter	Percent of the ear diameter attributablee to the cob (calculated from ear and cob circumferences at mid-ear)
Plant height	Height (cm) from the ground to the tip of the tassel central spike
Stalk height	Height (cm) from the ground to the uppermost leaf node
Early-season growth	Change in height (cm) from the ground to the tip of the longest leaf from 5 to 7 weeks post-sowing of the seed
Late-season growth	Change in height (cm) from the longest leaf tip at 7 weeks of age to the tip of the tassel central spike at maturity
Node number	Number of leaf nodes on the central stalk
Internode length	Average length of the internodal segments of the main stalk (calculated from the node number and the stalk length)
Days to silk	Number of days from sowing of seed to silk emergence on the uppermost ear
Ear height	Height (cm) from the ground to the node from which the uppermost ear was borne
Tassel branch number	Total number of primary and secondary tassel branches, excluding the central spike
Ear length/ diameter ratio	Ratio of the length to diameter of the upper ear

they represented a range of plant characteristics of agronomic significance. These traits are: ear height, ear number, days to silk, grain weight, plant height, kernel row number, kernel number, and tassel branch number. The marker loci in common for the two studies are: 9, 12, 15, 31, 48, 52, 68, 69, 71, 74, 76, 93, 104, and 110. Seventeen of 20 trait-locus combinations that were significant in this investigation also were significant and exhibited additive effects similar to those observed in the previous study. Thirteen of these exhibited exactly the same rank order of means for the three genotypic classes. Among all 112 comparisons examined, regardless of significance in either study, 83 exhibited similar additive effects and 55 exhibited exactly the same ranking of means in the two studies. This relatively high corroboration between the data sets is impressive, given that genotype × environmental interaction is a nonerror source of inconsistencies in the two studies, and that estimates made in this study were rather imprecise due to the small population size.

Taken in whole, these data provide a rather convincing argument that marker-facilitated investigations such as this one can be an effective means of elucidating factors influencing quantitative trait inheritance, even with rather small population sizes and in single environments. Because of the large confidence intervals with such small population sizes, further examinations allowing greater statistical power would be advisable prior to undertaking an effort to exploit any single, marker-linked factor. Although a proportion of the marker-linked quantitative trait loci are likely to have poorly estimated effects when estimates are determined using small population sizes, it is likely that net estimates of breeding values of plants across loci will be generally valid, despite inaccuracies at individual loci. Thus, greater confidence could be placed in the value of manipulating gene frequencies at a large number of the identified genomic sites influencing a particular trait (Edwards and Stuber 1986; Stuber and Edwards 1986).

Magnitudes of gene effects are represented here according to the portion of the total phenotypic variation in a quantitative trait that is "explained" by the marker locus (i.e., R^2), as was described by Edwards et al. (1987). The distribution of R^2 values across all traits and marker loci (not presented) was skewed, with a much greater frequency of associations producing a small R^2 value. The distribution was quite similar to those previously reported, except that the maximum R^2 values observed here were as great as 27.4% for locus 104 (*Acp1*) with PLTHT (compared to a previous maximum of 16.3% for *Idh1* with PLTHT). In the previous study, the *Acp1*-PLTHT association was not as great, with an R^2 value of 6%, although the ranking of mean values was identical. This locus-trait combination represents the most notable example of change of effects in the two studies. Previously, the magnitude of the *Idh1*-PLTHT association was more than twice as great as was the *Acp1*-PLTHT association (Edwards et al. 1987). In this investigation, the reverse was true.

In the previous investigation (Edwards et al. 1987), only 17 marker loci were employed to detect QTLs in this population. It was not possible, therefore, to separate the magnitude of gene effects at the underlying QTLs from effects attributable to recombination between the marker locus and the QTL (which would act to reduce the per-

Table 3. Relationship of plant height and related traits with marker-locus-linked genomic regions. Probability level of significant associations between each of 12 segregating marker loci and five traits related to plant stature, percent of the total variation ($R^2 \times 100$) accounted for by each marker locus, and parental homozygote (T = Tx303 and C = CO159) exhibiting the greater expression for that trait in (CO159 × Tx303) F_2

Plant-stature-related trait	Chromosome 1		Chromosome 2	Chromosome 3		Chromosome 4	Chromosome 6	Chromosome 7	Chromosome 8		Chromosome 9	Chromosome 10
	4	7	25	36	45	53	70	83	90	95	104	112
Plant height	**	*		*	*	***	*	*	***	***	***	*
	8.1	10.4	4.9	6.7	5.5	16.0	4.1	4.8	12.2	14.4	27.4	4.0
	T	T	T	C	C	T	C	T	T	T	T	T
Growth in height	***									*	**	
5 to 7 weeks	13.5	3.2	1.4	2.9	0.4	3.6	1.3	3.3	1.0	4.5	5.6	0.1
	T	T	T	C	T	T	C	T	T	T	T	T
Growth in height		*	*			***			***	***	***	*
7 weeks to	3.4	7.6	5.3	4.3	4.7	9.7	2.7	1.5	14.8	10.8	18.4	4.9
maturity	T	T	T	C	C	T	C	T	T	T	T	T
Internode			***			***		**		*	***	
length	0.3	0.6	11.5	3.8	3.2	16.3	0.9	6.6	3.9	4.1	20.0	2.6
	T	C	T	C	C	T	C	T	T	T	T	T
Node number	***	***	*			*	*		***	***	***	**
	16.7	25.6	5.8	4.5	4.4	4.0	3.8	0.5	17.2	17.9	10.1	5.9
	T	T	C	C	T	T	C	C	T	T	T	T

*, **, *** Denotes significance levels of *F*-tests at 0.05, 0.01, and 0.001 probability levels, respectively

ceived magnitude of effects at the QTL). With the large number of marker loci offered by RFLPs in this investigation, it was evident that some of the loci employed in the previous investigation were not very closely linked to the QTL producing the effect. Examples are the effect on leaf number associated with locus 9 (*Mdh4*) and the effect on ear height associated with locus 69 (*Enp1*). In both of these cases RFLPs approx. 25 cM from the isozyme locus exhibited associations of two to four times greater magnitude (based on R^2 values) than associations reflected at the isozyme loci.

Principle component analyses were employed in a previous investigation in an effort to simplify interpretation of the manifold effects of marker-linked genomic regions (Stuber et al. 1987). These analyses were somewhat unsuccessful, leading to the conclusion that individual genomic regions differed sufficiently in the range of their effects to preclude useful generalizations from principle component analyses. In this investigation, factors that affected final plant height again appeared to vary considerably in their effects on traits correlated with plant height (Table 3). Some genomic regions influenced plant growth early in development only (locus 4), others only affected growth later in development (loci 53 and 90), and some affected growth in both time periods (loci 95 and 104). Factors also varied in the components of plant height affected. Some affected node number but not internode elongation (loci 4, 7, and 90). Others apparently affected both characteristics (loci 95 and 104).

The effects of QTLs on grain yield and related traits appeared to be even more complex (Table 4). Some factors affected several yield "components" in a counterposing fashion, thus producing no net effect on grain yield (loci 25 and 78). QTLs were also detected that appeared to affect primarily a single yield-related trait, such as loci 39 and 44, which affected kernel row number only. Others, such as loci 5, 49, 78, and 94, influenced several yield-related characteristics.

A frequent observation in the previous investigation was the association of a single, marker locus with variation in a number of quantitative traits (Edwards et al. 1987). In fact, the average marker-linked region affected about two-thirds of the 82 quantitative traits analyzed. It is possible that these multiple effects are often attributable to multiple QTLs in the marker-linked genomic region, rather than being representative of pleiotropy. With only a single marker locus in a region, these two genetic possibilities are not resolvable in an F_2 population. In some circumstances in this investigation it was apparent that effects previously associated with a single marker locus are, in fact, attributable to more than one underlying factor. In the previous investigation, *Glu1* (locus 110) was associated with differences in 61 of the 82 traits examined. In this investigation, marker locus genotypes were established for five loci in the *Glu1* region of chromosome 10. Significant associations were again detected between markers in this region and a number of the traits previously examined (Fig. 3). However, three

Table 4. Relationship of grain yield (and related traits) with marker-locus-linked genomic regions. Probability level of significant associations between each of 21 marker loci and eight yield-related traits, percent of total variation ($R^2 \times 100$) accounted for by each marker locus, and parental homozygote (T = Tx303 and C = CO159) exhibiting the greater expression for each trait in the (CO159 × Tx303) F_2

Yield-related trait	Chromosomes 1				Chromosome 2				Chromosome 3			Chromosome 4			Ch. 6	Ch. 7		Ch. 8		Ch. 9	Ch. 10
	4	5	7	16	22	24	25	34	36	39	44	49	53	56	76	78	82	91	94	104	110
Grain weight	*	***	*	*		*			*			*	*	**	*			*	*	**	
	5.7	7.9	7.1	10.3	3.1	4.9	4.6	3.6	6.2	0.2	1.1	4.9	4.0	16.6	3.6	5.0	0.4	5.2	4.2	6.2	3.1
	T	T	T	C	T	T	T	T	C	C	T	T	T	T	T	C	T	T	T	T	T
Ear number	**	**			**												*		**	**	
	8.0	5.3	4.6	4.0	6.6	3.6	2.5	7.7	2.5	3.1	0.4	0.3	2.4	2.3	0.4	2.0	3.8	2.9	6.2	5.0	2.8
	T	T	T	C	T	T	T	C	C	C	T	T	T	T	T	C	T	T	T	T	T
Ear length		***			*	*	*								*				***		
	2.3	10.9	6.1	0.5	5.3	5.5	6.6	1.1	1.0	0.8	0.4	3.0	3.7	6.7	3.7	2.6	1.8	2.6	13.3	3.1	2.3
	T	C	T	C	T	T	T	T	C	C	C	T	T	T	T	C	T	T	T	T	T
Kernel depth								*				*		*							
	3.2	1.6	0.8	7.2	0.8	1.2	4.8	14.6	1.2	0.2	2.8	5.5	1.1	10.5	1.6	2.0	0.4	2.1	0.1	2.8	3.9
	T	T	T	T	C	C	C	T	C	T	T	T	T	T	T	C	C	C	T	T	C
Row number										**	*	**			***	*					
	0.1	0.1	1.3	4.3	0.0	0.2	4.0	3.9	0.8	12.5	8.9	5.9	0.2	9.0	10.1	8.9	1.3	3.3	1.3	2.8	0.8
	T	T	T	C	C	T	C	T	C	T	T	T	C	T	T	C	C	C	T	T	T
100-kernel wt.							*					***				**	**				
	0.6	0.5	1.0	2.4	0.8	2.7	6.3	1.0	4.7	0.6	1.3	8.2	0.0	4.7	1.7	14.4	7.7	2.1	2.4	0.6	0.5
	C	T	T	T	C	C	C	T	T	C	T	C	T	C	C	T	T	T	T	T	T
Kernel number	*	***		*		*			*			**		***	*	*			*	*	
	5.3	7.9	1.9	9.0	3.2	5.6	4.7	3.4	7.7	0.5	0.9	6.5	2.1	22.1	4.5	12.0	1.1	1.7	4.5	4.8	1.4
	T	T	T	C	T	T	T	T	C	C	T	T	T	T	T	C	C	T	T	T	T
% Cob diameter							*					**									*
	0.8	0.3	0.9	5.6	1.4	0.6	0.4	13.2	0.4	0.6	0.7	6.1	1.8	6.3	1.7	4.3	0.1	0.4	0.6	0.6	3.7
	T	T	C	C	T	T	T	C	C	C	C	C	C	C	C	T	T	C	T	C	T

*, **, *** Denotes significance levels of *F*-tests at 0.05, 0.01, and 0.001 probability levels, respectively

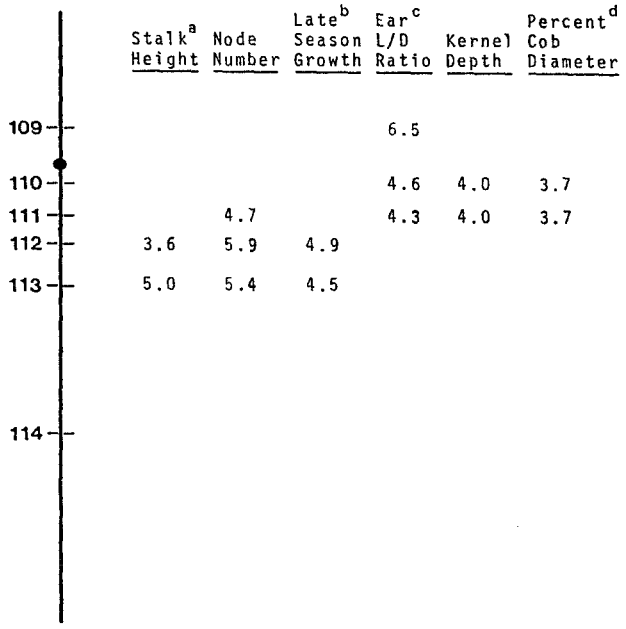

Fig. 3. Percent of the phenotypic variation (R^2) in six traits "explained" by linked marker loci on chromosome 10 in the (CO159 × Tx303) F_2 population. Variation in all six traits was significantly associated with *Glu1* (locus 110) in a previous investigation involving 1,776 F_2 plants from the same population. Nonsignificant R^2 values ($P > 0.05$) are not indicated. [a] height from the ground to the uppermost leaf node; [b] change in height from 7 weeks post-planting to maturity; [c] ratio of ear length to ear diameter; [d] cob diameter as a percent of total ear diameter

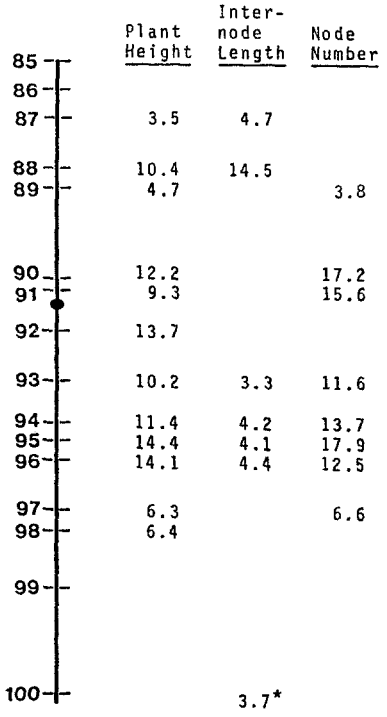

Fig. 4. Percent of the phenotypic variation (R^2) in plant height and two of its component traits "explained" by marker loci on chromosome 8 in the (CO159 × Tx303) F_2 population. Non significant R^2 values ($P > 0.05$) are not indicated. The *asterisk* indicates the factor for which the shorter parent, CO159, contributed the greater length

traits related to plant stature appeared to have effects centered more distally on the long arm of the chromosome (near loci 112 and 113) and three traits related to ear conformation appeared to be controlled by a factor(s) more proximal to the centromere.

As in the previous study, the loci *Idh1* (locus 93) on chromosome 8 and *Acp1* (locus 104) on chromosome 9 were associated with differences in a large number of traits: 12 of 30 and 14 of 30, respectively. Although other markers were assayed in both of these genomic regions, the effects on most of these traits appeared to be centered near these two isozyme loci rather than being centered on various flanking marker loci. These two genomic regions, then, must either exhibit pleiotropy or contain multiple, closely linked genes affecting subsets of these traits.

The availability of several marker loci in a given genomic region is, thus, a rather powerful tool for elucidating the nature of observed associations between marker loci and quantitative traits. This is particularly true in more advanced generations when linkage disequilibrium is reduced. In addition, the presence of multiple marker loci will expedite manipulation of QTLs by allowing selection for genotypes at flanking marker loci, thus greatly minimizing the risk that the desired factor may be lost due to recombination between the QTL and the markers employed in selection.

Soller et al. (1979) have argued from a theoretical perspective that there is usually only one, or at most two, QTLs affecting a given trait in a genomic region linked to a marker locus. It is difficult to address this issue empirically without multiple markers in each genomic region. Thus, the only evidence we could apply to this issue in our previous investigation was indirect. We frequently observed apparent overdominance at marker loci, particularly for grain weight and related characteristics (Edwards et al. 1987; Stuber et al. 1987). If true overdominance is assumed to be rare, then this often may have been attributable to repulsion-phase linkages of more than one partially dominant or dominant gene. In some circumstances in this investigation a much stronger case could be made for linkages of genes affecting a given trait. An example is illustrated in Fig. 4. Many of the marker loci on chromosome 8 exhibited associations with plant height. By examining two components of plant height, average internode length and node number, it became clear that factors in different regions of chromosome 8 influenced plant height via different means. The factor on the short arm, apparently centered near locus 88, primarily increased internode length and did not affect node number. The factor near the centromere affected node number, but not internode length. A major factor affecting plant height more distal from the cen-

tromere, near locus 95, appeared to act both through increases in node number and internode length. Finally, a "cryptic" factor from the shorter parent, CO159, apparently exists at the terminus of the long arm. This factor promoted internode length but did not affect plant height detectibly.

Most of these effects exhibited a progressive pattern in the magnitude of association with the linked marker loci (reflected as a peak in R^2 values), which suggested the most likely site of the QTL. The factor near the centromere appeared to exhibit this feature to a lesser degree (with respect to plant height, at least). Locus 92 was scored for only 62 of the 187 plants. There is, therefore, a rather large error associated with the R^2 at the locus, which may account for the irregularity in the pattern of the R^2 values in the region. From this example, it is apparent that rather sophisticated insights into gene effects for quantitative traits are possible with an extensive distribution of markers in the genome. This capability is not currently possible in most crop species with any type of marker other than RFLPs.

Another capability specific to RFLPs is the prospect of being able to proclaim, with some certainty, the number of factors (relatively minor factors perhaps excepted) that influence any given trait in some reference population. A substantial effort has been directed toward biometric means of inferring this sort of information (Mather and Jinks 1971; Baker 1984). Although this subject has produced abundant literature, there are serious limitations to the inferences that may be drawn from the biometrical methods employed in gene number estimates (Park 1977; Lande 1981; Baker 1984). Estimates obtained for some traits have been quite low, and must always be presented as "minimum estimates". With the rather complete coverage of the genome now available using neutral genomic markers, more reliable information on the issue of gene numbers for quantitative traits may soon be forthcoming.

The distribution of significant associations detected here for the traits plant height and grain yield are presented in Fig. 5. Eighteen and 12 chromosomal regions are implicated for the two traits, respectively. These numbers of factors are no greater than those detected in the same population in the previous study, which employed only isozymes as markers (Edwards et al. 1987). With the rather small population sizes employed, one must recognize that additional factors may exist but may produce effects too small to have been detected. However, the markers employed here should have provided a rather extensive coverage of the genome and should have detected most factors with fairly "major" effects. The variation in the magnitudes of the QTLs for each trait is notable. In addition, "cryptic" factors for both traits apparently come from CO159, the parent that is much smaller and yields a great deal less in North Carolina.

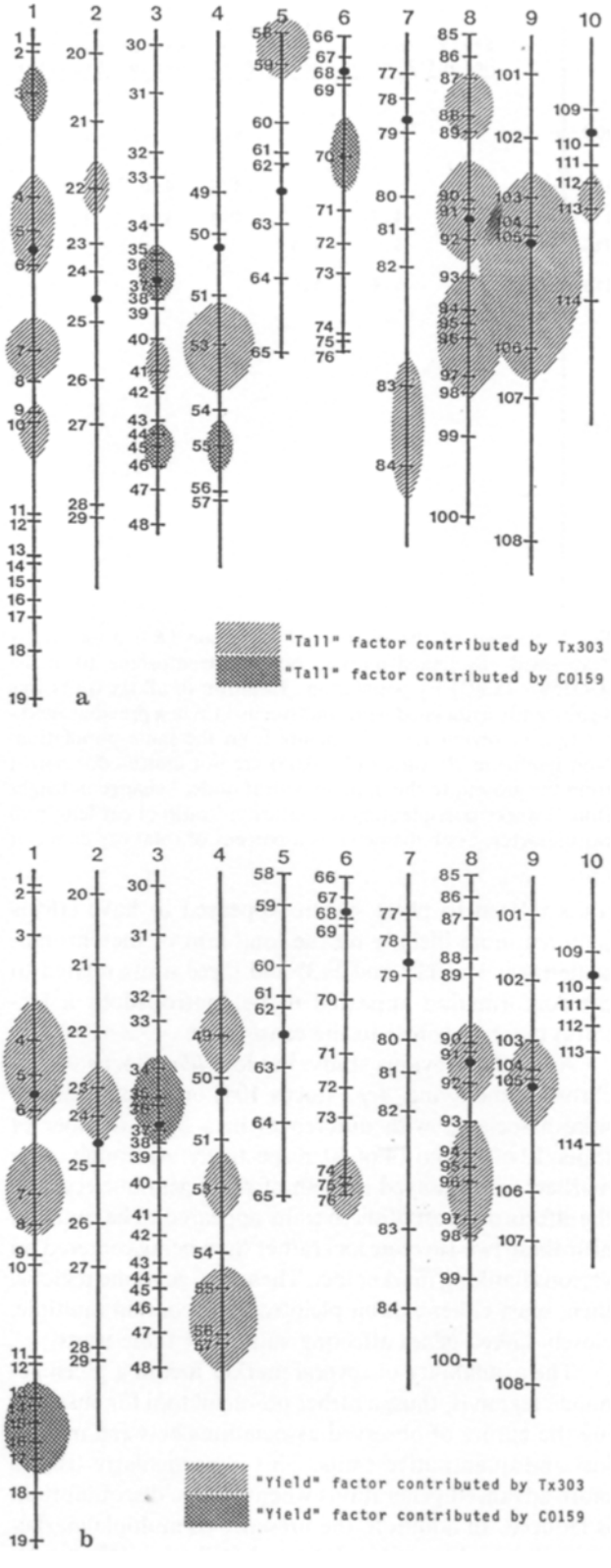

Fig. 5a, b. Approximate centromere locations and distribution of factors affecting *a* plant height and *b* grain yield in the (CO159 × Tx303)F_2 population. Relative magnitudes of the associated effects (R^2) are indicated for each trait by the widths of the shaded area in the marker-linked genomic regions

These QTLs are rather broadly distributed across the genome for both traits.

Although the genomic distribution of significant effects varied considerably from trait to trait, major factors influencing most of the 30 evaluated traits are not randomly distributed in this population. Chromosomes 1, 8, and 9 have a greater concentration of effects for most traits than do chromosomes 3, 5, and 7 (Table 5). It is interesting to note this apparent trend juxtaposed with recent evidence, which suggests that a rather close homology exists between portions of chromosomes 3 and 8 in maize, as well as some between chromosomes 1 and 3 and chromosomes 1 and 5 (Helentjaris et al. 1988). Of course, lack of segregation for quantitative effects in a particular genomic region in this F_2 population does not necessarily imply that important QTLs do not exist in the region. When information of this type is available in a much larger range of maize crosses, however, it will be interesting to determine whether a case can be made for widespread "silencing" or functional divergence of QTLs in duplicated genomic regions, as has been widely observed for duplicated qualitative loci (Ohno 1970; Ohta 1980).

As quantitative trait loci are detected and located, questions about their nature will surely receive some attention. Are they structural loci, regulatory loci, or are they even transcribed regions of the genome (Phillips 1986)? One alluring possibility is that some may be "minor" alleles at already known "major" gene loci (Thompson 1975). Although our data are not adequate to definitively test this possibility, we evaluated circumstantial evidence by comparing the locations of QTLs for plant height detected herein with the reported locations of major genes affecting plant stature (such as brachytic or dwarfing genes). A number of the mapped major genes that affect plant stature are located in the same genomic regions as factors detected in this investigation (Fig. 6). The figure indicates only the 17 mapped major genes for which the "primary" effects are associated with plant stature (Coe et al. 1990). There are probably many additional qualitative genes that might have been included, but that are either unmapped at present or are designated to indicate "primary" effects upon other traits. There is no reason to expect that all QTLs for plant height are alleles at major loci. Likewise, it need not be assumed that CO159 and Tx303 possess different alleles at all loci that affect plant stature. Obviously, further investigations will be required before sufficient evidence is available to support or disprove this possibility.

A highly saturated RFLP map provides a powerful adjunct to isozymes as a tool for determining the genetic architecture of quantitative traits in maize. Use of such a map has made it possible to determine the numbers, magnitudes, and locations of genetic factors underlying quantitative trait control, as well as insights into some complex genetic situations in specific chromsomal regions. RFLP-based investigations can provide definitive information about genetic determinants that have been individually unresolvable in the past for many agriculturally important characteristics of plants. Although the small population sizes employed herein produced QTL estimates with rather large confidence intervals, generally

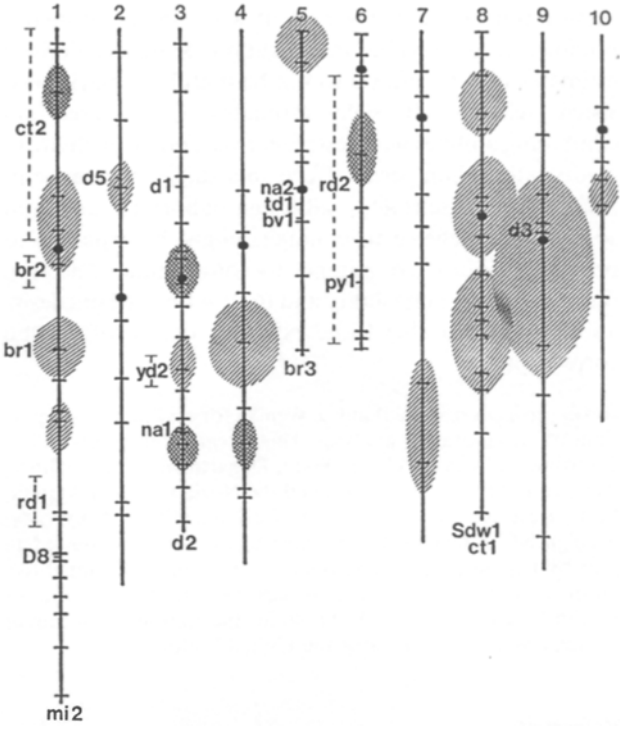

Fig. 6. Relationship between the chromosomal locations of mapped major genes reported to affect plant height in corn and marker-linked genomic regions having a quantitative effect on plant height in the (CO159 × Tx303)F_2 population. Centromere locations are approximate

Table 5. Distribution of significant associations between marker loci and 30 quantitative traits across the ten chromosomes in the (CO159 × Tx303) F_2 population. The average number of the 187 F_2 individuals that were assayed for genotypes at the marker loci on each chromosome is also indicated

Chromosome	No. of marker loci	Percent significant associations	Average N
1	19	20.0	144
2	10	11.0	121
3	19	7.9	131
4	8	17.1	127
5	8	7.9	136
6	11	10.6	165
7	8	7.5	142
8	9	25.0	144
9	8	26.3	145
10	6	12.2	161

these estimates agreed very well with similar estimates obtained in a previous investigation. Similarly derived estimates of QTL effects would be useful for marker-assisted selection if such estimates were based on combining abilities with testers across a range of environments rather than per se effects in a single environment. Marker-based selection, although laborious and still somewhat expensive to conduct, might be expected to provide an attractive adjunct to conventional selection for traits of low heritability and in situations where desirable and undesirable traits exhibit a degree of genetic correlation.

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