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Molecular marker-facilitated studies in an elite maize population: I. Linkage analysis and determination of QTL for morphological traits

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Abstract Restriction fragment length polymorphisms (RFLPs) and one morphological marker were used to investigate quantitative trait loci (QTL) for morphological and physiological traits evaluated on 150 $F_{2:3}$ maize (*Zea mays* L.) lines derived from the cross of elite U.S. Corn Belt inbreds Mo17 and H99. $F_{2:3}$ lines were grown in a replicated experiment and evaluated for plant and ear heights and flowering traits. QTL were identified for each trait, and genetic effects were determined. Estimated gene action for the flowering traits was predominantly overdominance. Both parents contributed toward increased values for anthesis and silk emergence. QTL for increased plant and ear heights were usually contributed by the taller parent, Mo17. Estimated gene action for these traits was mainly partial to overdominance. QTL for plant height were located in the vicinity of loci defined by alleles with qualitative effects on plant height.

Key words Maize · Restriction fragment length polymorphisms (RFLPs) · Qualitative and quantitative inheritance · Plant breeding · Genetics

Introduction

Marker-facilitated studies have been used to dissect traits into their genetic components. Sax (1923) demonstrated an association with seed weight and seed color in *Phaseolus vulgaris*. Further exploitation of this approach has awaited the development of more informative and efficient molecular marker systems.

The first readily available molecular marker system in plants was based on variation at isozyme loci. In maize, trait analyses of F_2 plants from two widely divergent populations identified significant associations between isozyme markers and 25 agronomic traits (Edwards et al. 1987; Stuber et al. 1987). To determine if quantitative trait loci (QTL) could be detected in more elite maize populations, Abler et al. (1991) used populations from six single crosses. Significant associations were detected for all traits. Up to 15% of the total phenotypic variation of a trait could be detected at a marker locus.

Restriction fragment length polymorphisms (RFLPs) have become widely used in the genetic analysis of quantitative traits of plants (Diers et al. 1992; Paterson et al. 1991; Yu et al. 1991). In maize, several quantitative traits have been investigated, and genomic regions controlling these traits have been identified by RFLP markers (Ottaviano et al. 1991; Reiter et al. 1991). Beavis et al. (1991) evaluated 112–144 $F_{2:4}$ progeny in each of four populations for plant height. Fourteen genomic regions were identified in the four populations, but only two QTL were common across more than one population. Three to six regions were identified in any one population, accounting for 34–73% of the phenotypic variation. Edwards et al. (1992) used 114 RFLP markers to evaluate 187 F_2 plants from the cross CO159 × Tx303. Eighteen traits were measured, including grain yield, yield components, plant height, and flowering. Single-factor analyses of variance detected significant associations with each trait.

Plant stature, anthesis, and silk emergence are high priorities of maize improvement programs (Hallauer 1990). These traits exhibit quantitative inheritance patterns and their genetic components typically have been characterized through biometrical methods based on estimates pooled over the entire genome (Hallauer and Miranda 1988). While QTL with major effects for plant height have been identified (Beavis et al. 1991), QTL for anthesis and silk emergence have not been reported from the analysis of replicated inbred progeny. If QTL could be identified, the transfer of these traits could be accelerated in breeding programs. Also, analysis of QTL for anthesis and silk emergence could identify novel genomic regions that have not yet been defined by alleles with qualitative effects; i.e., mutants. The objective of the study reported here was to identify genetic factors controlling plant stature and flowering in an elite maize population of replicated $F_{2:3}$ lines.

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Materials and methods

Population

The population was developed from a cross of adapted and widely used U.S. Corn Belt maize inbreds Mo17 and H99. Both of these inbreds are classified as members of the Lancaster Sure Crop (LSC) heterotic group according to their pedigrees and RFLP patterns (Melchinger et al. 1991), and they differ for several traits, including kernel size, plant height, flowering date, and grain yield (Russell et al. 1989). The cross was made in the 1988 breeding nursery near Ames, Iowa. Unselected F_2 plants were self-pollinated in the 1988–1989 Hawaii winter nursery to produce 150 $F_{2:3}$ lines.

Field design

The lines were evaluated in a 12 × 13 simple rectangular lattice design of one-row plots with two replications at the Agronomy and Agricultural Engineering Research Center near Ames in 1989. The rows were 5.5 m long with 0.76 m spacing between rows. Plots were machine planted at a density of 76,540 kernels ha^{-1} on April 20, 1989 and were thinned at the six- to eight-leaf stage to a density of approximately 57,400 plants ha^{-1}. Fertility and cultivation regimes were consistent with optimum maize production for this region. The months preceding and during the growing season at Ames had below normal precipitation (seventh driest year on record) and were preceded by a drought at the location in 1988 (the driest year on record). However, below normal temperature and timely rains during pollination permitted complete growth and development.

The entries consisted of 150 $F_{2:3}$ lines plus six entries of a bulk of all 150 lines. Flowering dates and plant and ear heights were measured on a plot basis. Accumulated growing degree days (GDDs) in °C to anthesis were calculated from date of planting to the date 50% of the plants in a plot had exerted anthers. GDDs for silk emergence were measured from date of planting to the date 50% of the plants in a plot had silks emerged from the primary ear shoot. Silk delay was calculated on a plot basis as the difference between GDD to silk emergence and GDD to anthesis. GDD were calculated according to the formula: [(maximum °C + minimum °C)/2] − 10 °C, where 10 °C was used for the minimum temperature and 30 °C used for the maximum temperature if the actual temperatures exceeded these limits. After all of the plants had completed anthesis, mean plant and ear heights for each plot were determined from 5 competitive plants measured to the nearest 5 cm. Plant height was measured from the soil level to the tip of the central tassel spike, and ear height was measured from the soil level to the node of attachment of the primary ear.

RFLP assays

Twelve kernels of each $F_{2:3}$ line, Mo17, H99, and the F_1 were planted in the greenhouse. An equal quantity of fresh leaf tissue from an average of 9–10 plants were harvested at the five- to six-leaf stage for each line. For 10 lines, only 6 plants were available, which still assured the probability to be greater than 99.9% of representing both alleles at a segregating locus (Hanson 1959). The tissue was placed in a lyophilizer for 5–7 days ground to a fine powder, and stored in vials at −20 °C.

Genomic DNA was isolated based on the CTAB method (Saghai-Maroof et al. 1984) from 0.7 g of ground tissue. Three sets of single-enzyme digests of the DNA were made with the enzymes *Hin*dIII, and *Eco*RI, *Eco*RV by using 10 u enzyme/μg of DNA in 1 × manufacturer's buffer and 25 m*M* spermidine for 4 h. Digested DNA (10 μg/lane) of parental, F_1, and $F_{2:3}$ lines was loaded in 0.7% agarose gels, electrophoresed, and transferred to nylon membranes. The probe (100 ng) was radiolabeled by using the random-primer method (Feinberg and Vogelstein 1983). Membranes were prehybridized in 6 × SSC, 0.5% SDS, 1 × Denhardt's 25 m*M* $NaPO_4$, and 100 μg/ml denatured herring sperm DNA at 65 °C for 4 h (15 ml solution/1,000 cm^2 of membrane). The probe was added to the prehybridization solution, and hybridization occurred overnight. Membranes were rinsed twice with wash solution (100 ml of 0.1 × SSC, 0.1% SDS) followed by five 1-h washes using 100 ml of wash solution at 65 °C. The membranes wrapped in cellophane were exposed to Kodak XAR-5 film using two intensifying screens at −80 °C for 5–7 days $F_{2:3}$ lines on the autoradiographs were classified for each probe according to the banding patterns of the parental and F_1 lanes. Autoradiographs were scored twice and independently verified.

RFLP probes were provided by Native Plants Inc (npi) (Weber and Helentjaris 1989), Brookhaven National Laboratories (bnl) (Burr et al. 1988), University of Missouri-Columbia (umc) (Coe et al. 1990), and Pioneer Hi-Bred International (php) (Beavis and Grant 1991) or were from our laboratory (isu). Three markers derived by *Mutator* transposon-tagging of defective kernel (*dek*) mutants (Lee et al. 1991) were mapped in this population: *emp2* on 2L, *ren1* on 5L, and *ren2* on 7L. Other clones mapped in this population include *Agp1*, *Agp2*, *sh2*, and *bt2* (C. Hannah, University of Florida); *bt1* (O. Nelson, University of Wisconsin); and *Pl1* and *C1* (K. Cone, University of Missouri). These clones of morphological markers mapped to their expected locations (Coe et al. 1990). One morphological marker, red pigment in the cob (*P1*), was also included in the linkage analysis. In a preliminary survey of the inbred parents, probes detecting clear, low-copy number, codominant polymorphisms with a given enzyme were chosen based on their distribution on previously published maps. After the initial map was made, probes were added to improve coverage of the genome. This produced a final map with 103 loci defined by RFLP markers.

Data analysis

Trait data was analyzed with PLABSTAT (Utz 1987). Entry means adjused for incomplete block effects in the lattice design and effective error mean squares were calculated as described by Cochran and Cox (1957). The sums of squares for entries were partitioned into sums of squares due to $F_{2:3}$ lines and checks.

Variance components for σ^2 (effective error variance) (Cochran and Cox 1957) and σ_g^2 (genotypic variance) of the lines and the standard error (SE) of genotypic variance were calculated (Searle 1971). Broad-sense genetic determinations (heritability) of traits and their standard errors were calculated on the basis of formulas by Hallauer and Miranda (1988) for one environment. Simple Pearson phenotypic correlations (r_p) were calculated for each trait using the means of the 150 $F_{2:3}$ lines.

Deviations from normality

The $F_{2:3}$ line means had highly significant deviations from normal distributions on the basis for the Shapiro and Wilk (1965) W statistic for GDD to anthesis, GDD to silk emergence, and silk delay. Normality of distribution is one of the assumptions of interval estimations used in MAPMAKER-QTL (Lander and Botstein 1989). Because no transformations to normalize the data could be found, the data were used as recorded with the realization that a decrease in sensitivity and ability to estimate effects could result. Previous studies having non-normal distributions of traits have been reported (Doebley et al. 1990; Paterson et al. 1991; Knott and Haley 1992).

Linkage analysis

Chi-square analyses were performed on each probe to detect deviations from the expected Mendelian segregation of a 1:2:1 ratio.

The genomic composition of $F_{2:3}$ lines was determined by summing the number of loci representing each marker class of a line and dividing by the total number of loci scored for that line. This is a more conservative estimate than the method used by Paterson et al. (1988), and it does not assume specific recombinations.

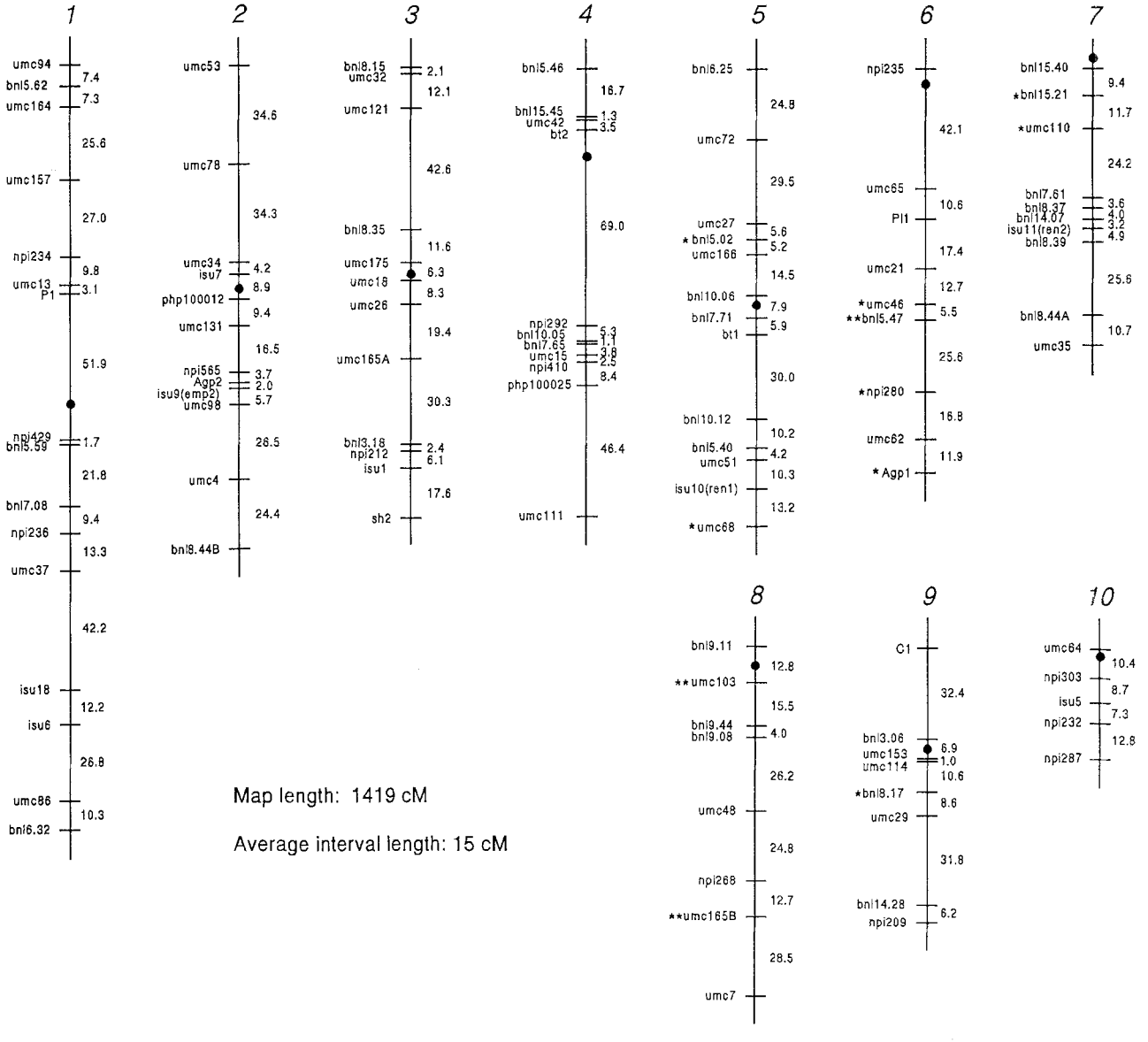
MAPMAKER (Version 1.9, Lander et al. 1987) was used to create the linkage map of the 93 random RFLP probes, ten clones of genes, and one morphological marker, P1, for the population of $F_{2:3}$ lines. Linkage was declared when a LOD threshold of 3.0 and recombination frequency of 0.40 were met.

QTL likelihood determinations

QTL likelihood plots (Paterson et al. 1988) were constructed for each trait using MAPMAKER-QTL (Version 0.9). For the analysis presented in this paper, a LOD threshold of 2.0 was used to declare the presence of a putative QTL in a given genomic region. Using the "sparse-map" case, this corresponds to an effective probability of $P < 0.10$ (given 94 marker intervals) that a false positive is declared in the entire genome (Lander and Botstein 1989). This is a lower level than used in previous studies; however, this level was chosen to reduce Type II errors. Further testing with more environments and samples of the population may resolve estimates of QTL positions. A LOD threshold of 2.4 would be needed to test at the 0.05 level of significance according to the Lander and Botstein (1989) "sparse-map" case. When more than one peak occurred within a linkage group, the peak with the largest LOD was 'fixed' (Lander and Botstein 1989), and a QTL likelihood plot was recalculated; i.e., the second peak was reexamined in a model that already accounted for the effects of the first peak. If the LOD for the combined model was significantly higher, two QTL are likely.

Genetic effects and percentage of phenotypic variation attributable to individual putative QTL were estimated at the peaks of significant regions when the genome was evaluated by using the unconstrained genetic model. Because $F_{2:3}$ progeny were used for trait evaluation, the estimates of dominance effects of genotypic means are expected to be reduced by half from heterozygous F_2 plants. In this instance, estimates of dominance effects were doubled in accordance with established procedures (Mather and Jinks 1971). Average levels of dominance were calculated as the ratio d/a with the dominance effects, d, being the dominance effects estimated for the F_2 population. Gene action was determined based on the average level of dominance by using the criteria of Stuber et al. (1987): additive (A) = 0–0.20; partial dominance (PD) = 0.21–0.80; dominance (D) = 0.81–1.20; and overdominance (OD) > 1.20. Because the effects of the QTL are being estimated in the same experiment in which the QTL were found to be significant, it is realized that the effects will be biased upwards (Lande and Thompson 1990). Additionally, the total percentage of variation accounted for in each trait was determined in a multiple model that included all of the significant QTL (Lander and Botstein 1989).

Fig. 1 RFLP map of Mo17 × H99 $F_{2:3}$ lines; loci with distortions from Mendelian segregations are marked with * and ** for 0.05 and 0.01 levels of significance, respectively



Results

Linkage analysis

The 104 markers produced the linkage map in Fig. 1. Some regions do not have complete coverage, which may be due to a lack of polymorphic markers or to high levels of recombination. For example, all attempts to identify loci in the 69-cM interval on chromosome 4 continued to map at the boundaries of this region even though estimates of genetic distances are much less in other populations. Due to the lack of loci in this interval, linkage could not be established; therefore, it was pieced together based on previously published maps. No QTL determinations were made in this interval. Also, relatively sparse coverage was obtained for regions of chromosomes 7, 8, 9, and 10. The placement of centromeres was based on maps of Coe et al. (1990).

Segregation analysis of marker loci

At 93 loci, parental and heterozygous classes fit expected Mendelian ratios of 1:2:1, although 11 loci had greater deviations than expected at the 0.05 or 0.01 levels of significance (Fig. 1). On chromosome 6, 3 adjacent loci (umc46, bnl 5.47, and npi 280) had a much greater frequency of heterozygotes associated with a decrease of homozygotes for H99 alleles. Also, homozygotes for the Mo17 allele were deficient at the bnl 5.47 locus. On chromosome 7, genotypic classes at bnl 15.21 and umc 110 had a deficiency of homozygous Mo17 genotypes and an excess of heterozygotes. Distortions at 5 other loci were detected on chromosomes 5, 8, and 9. These loci were unlinked and have a higher than expected proportion of the Mo17 or the H99 homozygous class. If, however, an experiment-wide error rate of 0.05 is considered for the 104 markers, only bnl 5.47 had significant deviations.

Five loci had significant deviations (at the 0.05 level of significance) from the expected allelic frequency of 1:1. These include 2 linked loci, isu 18 and umc 86, on 1 that had greater than expected frequencies of H99 alleles. The other 3 loci, bnl 5.02 on 5, umc 103 on 8, and bnl 3.06 on 9, were unlinked and had a greater than expected frequencies of Mo17 alleles.

Genomic composition

The average genomic composition was 24.3% homozygous Mo17, 22.8% homozygous H99, and 52.9% heterozygous (Fig. 2). The standard deviations were 9.5%, 8.4%, and 10.8%, respectively, which places the genotypic class proportions within the frequencies expected for $F_{2:3}$ lines. The distribution of genomic composition among lines ranged from 1.0% to 45.5% homozygous Mo17, 7.3% to 44.7% homozygous H99, and 23.1% to 84.9% heterozygous.

Field trait analysis

Means of plant and ear heights for the $F_{2:3}$ lines fit normal distributions. Deviations from normality were detected for the flowering traits and were most likely caused by the lack of precipitation and reduced soil moisture reserves at the beginning of the season. These conditions typically extend the time required to reach anthesis. Two-thirds of all the entries flowered in a short period (767–811 GDD for anthesis and 778–844 GDD for silk emergence) likely coinciding with rain. Most of the remaining entries flowered over a period of 111 GDD after this group. The timing of the rainfall probably caused the observed distribution for anthesis and silk emergence.

Genotypic variances of the $F_{2:3}$ lines were significant for all traits (Table 1). Broad-sense heritability estimates were high

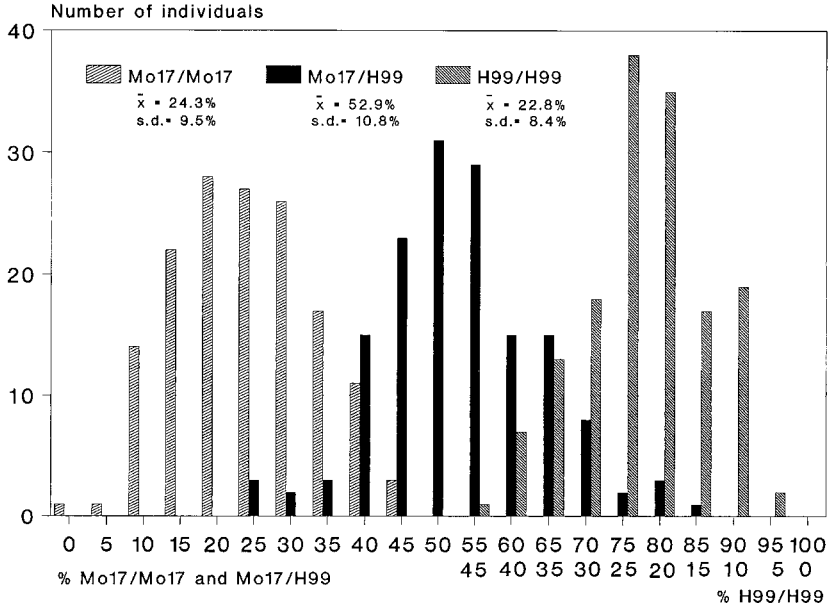


Fig. 2 Frequency distributions of the 150 $F_{2:3}$ lines for percentage homozygosity at each parental class (Mo17/Mo17) and (H99/H99) and percentage heterozygosity (Mo17/H99)

for all traits, but these were biased upwards because there was no estimate of the genotype × environment variance component. Anthesis, silk emergence, and silk delay exhibited transgressive segregation, and the means of all $F_{2:3}$ lines for anthesis and silk emergence were earlier than the midparent values for those traits. Heterosis was evident for plant and ear heights with the average of all the $F_{2:3}$ lines being greater than the high parent (Mo17) mean for plant height and higher than the midparent mean for ear height.

The phenotypic correlation between plant and ear height was highly significant ($r_p = 0.83$), while the phenotypic correlations between plant height and flowering traits were not significantly different from zero. Ear height was correlated with anthesis ($r_p = 0.33$) and silk delay ($r_p = -0.25$), but the coefficients were low. Highly significant phenotypic correlations were observed between anthesis and silk emergence ($r_p = 0.77$) and between silk emergence and silk delay ($r_p = 0.64$), but a significant correlation was not found between anthesis and silk delay ($r_p = 0$).

Analysis of traits for QTL

Six unlinked genomic regions had significant effects on anthesis (Table 2). Mo17 and H99 each contributed three regions for increased GDD to anthesis, and these regions accounted for 6.2–33.6% of the phenotypic variation. The multiple QTL model accounted for 62.9% of the variation for anthesis in this population. Five unlinked regions were detected for GDD to silk emergence (Table 2). Percentage of phenotypic variation explained by individual QTL ranged from 7.8% to 53.1%, and the multiple QTL model accounted for 80.9% of the phenotypic variation. Four of the regions controlling silk emergence are regions that influence anthesis, 1L, 6L, 7L, and 8L (Fig. 3). The region on 1L for increased GDD of both traits was devired from Mo17. On 6L, H99 contributed to increased GDD in both traits. However, on 7L, alleles from Mo17 increased GDD to silk emergence, but H99 increased GDD to anthesis. Both QTL exhibited overdominance towards reducing the GDD of their respective traits in this region. Two significant unlinked regions were detected for silk delay and collectively accounted for 30.9% of the variation (Table 2). Both regions correspond with QTL that affected anthesis and silk emergence. For these three traits H99 alleles contributed to increased values.

Five genomic regions were significantly associated with ear height (Table 2), and collectively these accounted for 61.8% of the phenotypic variation. In 4 regions, Mo17, the parent with the higher ear placement, contributed to increased ear height. H99 had 1 region, on 7L, that contributed to increased ear height, but it had the smallest effect. The QTL on 1L gives an increase of 10.7 cm for each Mo17 allele; i.e., an estimated difference of 21.4 cm between the parental classes. Plant height had 5 significant regions accounting for 67.1% of the variation. These included 3 putative QTL that seemed to correspond with QTL for ear height (Fig. 3), a highly correlated trait ($r_p = 0.83$). The 3 QTL for each trait also have the same parent contributing the direction of response: Mo17 on 1L and 6L, and H99 on 7L. The QTL on 1L, acting alone in an additive manner, accounted for 39.5% of the variation, a difference of 30.2 cm between parental classes. Of the 5 regions 3 have Mo17 alleles contributing to increased plant height. The 2 regions with the smallest effects were derived from H99.

Discussion

Linkage analysis in $F_{2:3}$ population

Genomic coverage and composition

The genomic compositions of the 150 lines (Fig. 2) fell within the expected frequencies and were similar in their means and

Table 1 Means, genetic variance component estimates, and heritabilities of $F_{2:3}$ lines for GDD to anthesis and silk emergence, silk delay, and plant and ear heights

	Trait				
	Anthesis	Silk emergence	Silk delay	Ear height	Plant height
	GDD	GDD	GDD	cm	cm
Means	°C	°C	°C		
Mo17[a]	854	909	55	80	167
H99[a]	791	802	11	34	103
$F_{2:3}$ lines	795	829	34	73	209
Range					
$F_{2:3}$ lines	731–904	763–951	2–119	40–119	161–251
Variance components ($F_{2:3}$ lines)					
$\sigma_g^2 \pm$ SE	603 ± 76**	976 ± 127**	345 ± 52**	181.5 ± 23.5**	243.3 ± 32.5**
σ^2	114	247	195	42.1	73.4
Heritability ($F_{2:3}$ lines)					
h^2	0.91	0.89	0.78	0.90	0.87
90% C.I. on h^2[b]	(0.91, 0.93)	(0.85, 0.92)	(0.71, 0.83)	(0.86, 0.92)	(0.83, 0.90)

** Significance at 0.01 level

[a] Mo17 and H99 data obtained from the 1989 Iowa State University Experimental Corn Trials (Russell et al. 1989) grown at the same location and planted April 24, 1989

[b] Knapp et al. (1989)

Table 2 Genomic locations, genetic effects, and percentage of phenotypic variation for QTL of morphological traits

Chromosome region	Nearest RFLP locus	Distance[a] (cM)	Maximum LOD score	% Variation	Genetic effects[b]			Gene action[c]	Direction[d]
					a	d	d/a		
GDD to anthesis					GDD				
1L	umc37	14	5.66	30.8	−17.1	−34.5	2.02	OD	Mo17
5L	bnl10.12	0	2.38	7.0	−2.8	−25.6	9.04	OD	Mo17
6L	bnl5.47	12	5.85	33.6	17.8	−47.6	−2.67	OD	H99
7L	bnl15.21	−3	2.64	8.9	3.7	−28.2	−7.70	OD	H99
8L	umc165B	14	3.15	18.8	16.2	−22.2	−1.37	OD	H99
9L	bnl14.28	0	2.10	6.2	−8.5	−10.8	1.27	OD	Mo17
Total[e]			19.06	62.9					
GDD to silk emergence					GDD				
1L	umc37	12	7.66	36.1	−24.5	−47.7	1.94	OD	Mo17
2L	umc4	6	2.49	10.9	15.1	−8.4	−0.56	PD	H99
6L	bnl5.47	12	11.27	53.1	28.8	−81.2	−2.82	OD	H99
7L	bnl15.21	−1	2.40	7.8	−4.0	−37.4	9.35	OD	Mo17
8L	umc165B	14	7.40	39.4	29.2	−54.1	−1.85	OD	H99
Total			26.15	80.9					
Silk delay					GDD				
6L	bnl5.47	12	2.80	17.5	9.7	−29.6	−3.04	OD	H99
8L	umc165B	14	3.20	15.8	11.1	−25.2	−2.27	OD	H99
Total			5.90	30.9					
Ear height					cm				
1L	umc37	20	5.31	27.8	−10.7	−5.6	0.52	PD	Mo17
3S	bnl8.15	0	2.46	7.3	−5.5	−2.6	0.47	PD	Mo17
5L	bnl5.40	−4	2.10	7.0	−3.9	−9.9	2.54	OD	Mo17
6L	npi280	8	4.84	17.3	−8.6	10.7	−1.24	OD	Mo17
7L	isu011(ren2)	0	2.11	6.3	5.3	1.4	0.26	PD	H99
Total			20.76	61.2					
Plant height					cm				
1L	umc37	18	8.21	39.5	−15.1	−1.0	0.07	A	Mo17
2S	umc34	−14	2.18	9.3	−6.9	−8.7	−1.26	OD	Mo17
4L	bnl15.45	0	2.16	6.4	5.7	9.3	1.63	OD	H99
6L	npi280	−8	4.44	17.0	−10.7	12.5	−1.17	D	Mo17
7L	umc35	−3	2.60	8.8	6.1	−13.7	−2.25	OD	H99
Total			21.26	67.1					

[a] The distance is measured from the nearest RFLP marker to the maximum LOD peak of a QTL. A positive distance is given for QTL located toward the terminal end of the long arm of the chromosome from the marker, and a negative distance is given for QTL located toward the terminal end of the short arm

[b] Additive effects are associated with the allele from H99. Therefore, a negative value means that the H99 allele decreases the value of the trait

[c] Gene action is determined from the ratio d/a

[d] Direction of response is the parent whose additive value of a marker allele increased the value of the trait

[e] Totals are the LOD score and the percentage of phenotypic variation accounted for in the multiple QTL model

ranges to those observed by Paterson et al. (1991) in an interspecific tomato population. Such information on the composition of lines could have immediate utility in plant breeding programs. This variability among lines can be used to a breeder's advantage to accelerate the recovery of recurrent parents in backcrossing programs (Young and Tanksley 1989) and to identify lines with the greatest number of homozygous loci at early generations of inbred line development (Paterson et al. 1991). In our analysis, an intraspecific cross between two elite maize inbreds clearly illustrated the possibility of recovering lines more closely resembling one parent, despite the small sample size.

QTL identified for morphological traits

Effects of identified QTL

Five to six QTL were identified for each trait with the exception of silk delay, which had only 2 QTL detected. However, silk delay had a high ratio of experimental error to the mean (coefficient of variation = 41%) and had the greatest distortion from normality, which could limit our ability to attribute variance to QTL. Only QTL with relatively large effects (> 15% of the phenotypic variability) were detected for silk delay. The smallest amount of variation accounted for by a

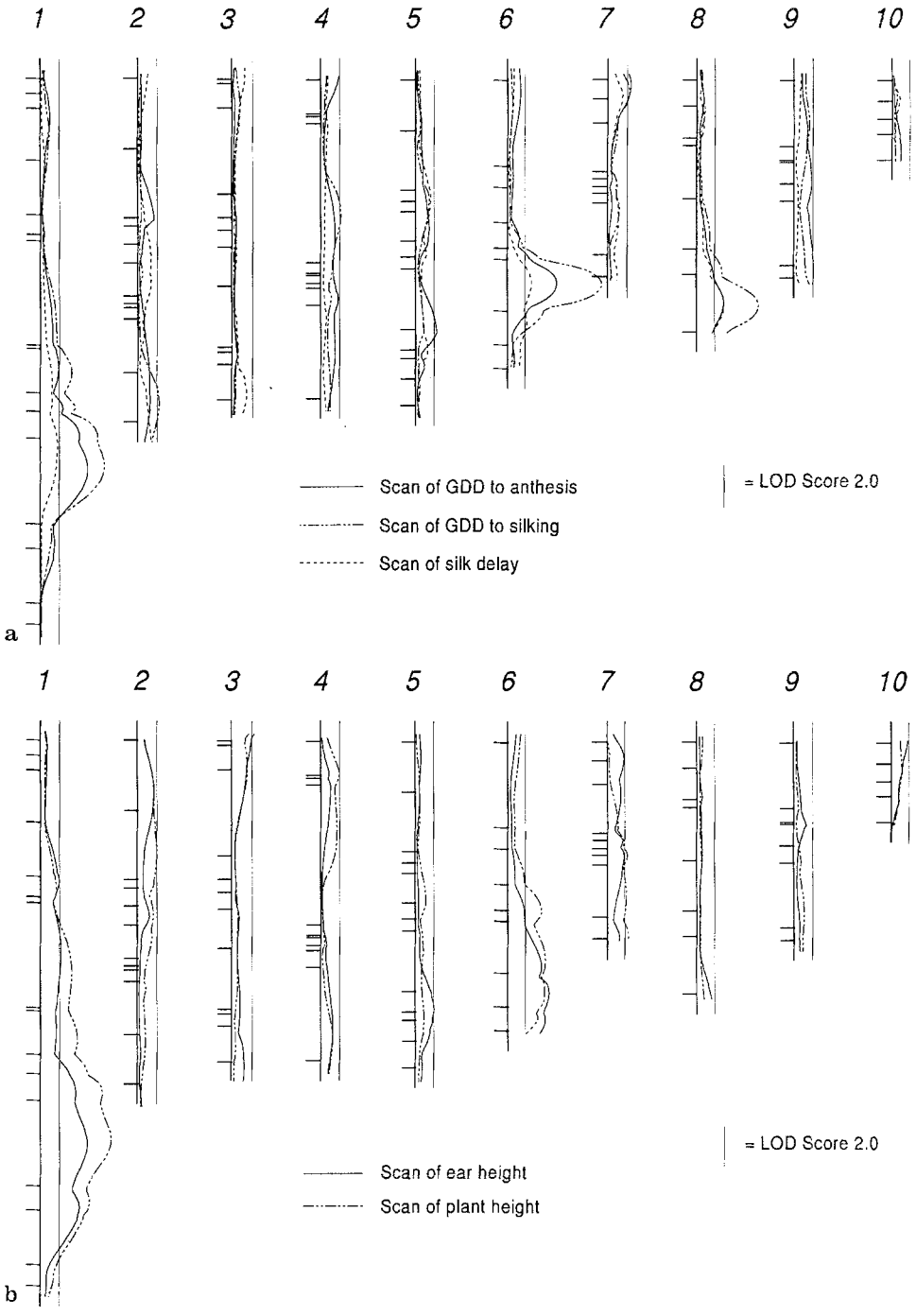


Fig. 3a, b **a** QTL likelihood plots of flowering traits; **b** QTL likelihood plots of ear and plant heights

single QTL was 6.2% to 7.8% for each trait, with the exception of silk delay. This amount seems reasonable when compared with other studies using larger sample sizes. Edwards et al. (1987) reported markers accounting for only 0.3% of the variation in populations 12 times larger than this population. Doebley et al. (1990) were able to detect 4% of the variation using a population from a maize × teosinte cross that was almost 2 times as large as this population.

The magnitude of the additive effects, or breeding value, of some putative QTL was suprising. For plant height, a QTL on 1L accounted for almost 40% of the phenotypic variation. The mean of the parental marker classes for this QTL differed by 30 cm. QTL with effects of this size (> 25% of variability explained in a single chromosomal region) were observed for all traits except silk delay. Silk emergence has 4 genomic regions, which explains a large proportion of the phenotypic variation individually. The QTL on 6L accounts for 52% of the phenotypic variation of anthesis, a difference of 59 GDD between the parental classes. Typically, that is a difference of a minimum of 3–4 days during July at this location.

The total amount of phenotypic variation detected by QTL ranged from 31% for silk delay to 84% for silk emer-

gence. The amount detectable was limited by the heritability (the ratio of the genetic variance to the phenotypic variance) of the trait. For silk emergence, the value was very close to the estimated heritability (89%). Therefore, any undetected QTL would likely only account for a very small amount of the variation. For silk delay, less than half of the heritability was explained by markers.

Pleiotropic effects

Pleiotropy was suggested by our observations of several genomic regions. The region on 1L near umc 37 had a large effect on anthesis, silk emergence, and ear and plant heights. Mo17 contributes to an increase in each of these traits in this region. The genomic region defined by bnl 5.47 and npi 280 on 6L influenced all of the traits, with Mo17 contributing to increased values for each trait. QTL for flowering traits were determined at the same position (Table 2) and have similar QTL likelihood plots (Fig. 3a); these were very different from the QTL likelihood plots of plant and ear height in the same region (Fig. 3b). This might indicate that the flowering traits are controlled by the same QTL, which in turn, differ from QTL controlling plant and ear heights in that region. A similar response for flowering traits was observed in the region on 8L with H99 contributing to increased values for these traits. To determine if the flowering and plant height traits were controlled by 2 or more linked genes or by the pleiotropic effects of 1 gene, an analysis would need to be made using a greater number of linked markers, an increase in the number of progeny evaluated, and possibly random mating to increase recovery of recombinants.

Correlation of traits

Paterson et al. (1991) and Abler et al. (1991) have shown that correlated traits often have some of the same markers significantly associated with each trait. The common genetic basis of some correlated traits was indicated in this population. Plant and ear heights have a high correlation ($r_p = 0.83$) and seem to have 2 QTL defined by the same marker intervals. There also seems to be substantial similarity in the QTL likelihood plots for these two traits, including regions that did not exceed the LOD threshold (Fig. 3b). Anthesis and silk emergence have 4 genomic regions in common that affect the trait, and these traits have a high correlation coefficient ($r_p = 0.77$). Common QTL also occur for the other correlated traits in this population. Phenotypic correlations were not observed for all traits with QTL defined at common loci. For example, the 2 QTL for silk delay have been identified by the same loci that define QTL for anthesis and silk emergence. Phenotypically, silk delay and emergence were highly correlated, but silk delay was not correlated with anthesis. Another example is plant height and the flowering traits. These traits were not significantly correlated, yet they each have QTL in corresponding regions on 1L and 6L that explain a large portion of the phenotypic variation.

Gene action and direction

Gene action for plant and ear heights showed mainly partial dominance to overdominance. For the flowering traits, gene action showed almost exclusively overdominance. Factors controlling anthesis and silk emergence were located on 7L near bnl 15.21. Here, Mo17 contributed to later silk emergence, whereas the H99 alleles contributed to later anthesis. The observation of substantial overdominance for each trait (7.7 for anthesis and 9.3 for silk emergence) in this region and the contribution of additive effects by different parents for each trait suggests that 2 or more QTL may be linked in repulsion. If the QTL each affect both traits, which is possible because they are both flowering traits, this could be an example of 'pseudo-overdominance' in which the heterozygote mimics overdominance and the additive effects partly nullify each other when the genes are homozygous and in repulsion (Moll et al. 1964). If these are QTL linked in repulsion and if linkage equilibrium were obtained, then the expected additive effects would be increased and the dominance effects decreased for each trait. Because almost all QTL for flowering traits and many QTL for plant and ear heights display overdominance, other cases of repulsion linkage could exist in this population.

For plant and ear heights, the direction (i.e., which parent contributes to increased values for the trait) and amount of response were as expected. Mo17, the taller parent, contributed the largest effect to increased plant and ear heights. H99 did contribute to height at a few loci, but these loci had the smallest effects. Three of the regions for plant height corresponded with regions reported by Beavis et al. (1991). The peaks on 1L and 2S occur near markers umc 37 and umc 34, respectively, which were located within the support intervals in the B73 × G35 population, and the peak on 4S near locus bnl 15.45 was located in the support interval in the B73 × Mo17 population. The QTL on 1L and 2S may be similar in position as those reported by Edwards et al. (1992), although direct comparisons were not possible.

For the flowering traits, the direction of response was not in good agreement with expectations based on the phenotype of the parents. The earlier flowering parent, H99, had large effects on delayed anthesis and silk emergence and on increased silk delay (Table 2). This was surprising because Mo17 requires more GDD for anthesis and silk emergence and expresses delayed silk emergence. For silk delay, when the 2 QTL were considered in a single model, there was a 36 GDD difference between the parental classes (data not shown), with the H99 parental class having the greatest amount of silk delay. The influence of the H99 alleles was unexpected because Mo17 has a 44 GDD greater silk delay than H99 when evaluated as pure lines (Table 1). This unexpected source of variation could be attributed to epistasis. Favorable (or unfavorable) alleles could be present in an inbred but not expressed in the inbred's genetic background. When crossed to another inbred, however, epistatic interactions could affect the expression of these alleles.

In our population, dominance deviations decreased the GDD at every QTL of the flowering traits (Table 2). This was true regardless of which parent contributed to increasing the

trait at a given locus. Heterosis towards earlines also was observed in the $F_{2:3}$ lines (mean of lines less than midparent value) (Table 1). The observation that earlier flowering is dominant to lateness for temperate region maize is in good agreement with the experience of maize breeders (Hallauer 1990).

Transgressive segregation

$F_{2:3}$ lines with trait values exceeding parental extremes, transgressive segregants, were observed for all traits. This phenomenon has been typically observed by plant breeders on many occasions, yet a genetic basis has not been determined. In our population, it seems that transgressive segregation could be due, in part, to unexpected genetic contributions. For example, H99 has reliably exhibited earlier flowering and good synchrony between anthesis and silk emergence; however, this parent contributed QTL for later flowering and silk delay. On the basis of such observations, it should be possible to recover progeny from this population that contain alleles at QTL from H99 and Mo17 that would exceed parental performance for the traits of interest.

Environmental effects

Studies such as this one will help elucidate the complexities of the maize genome. Previous studies have shown that a unique array of QTL may be detected in different environments and populations, with some QTL being stable across environments (Paterson et al. 1991; Schön et al. 1993) and populations (Beavis et al. 1991). Stuber et al. (1992) demonstrated a consistency of maize QTL across environments and suggested that major QTL could be reliably detected in few environments. We report results from one environment with the understanding that these traits are influenced by the environment. Further research using more environments and additional samples of this population will be compared.

Relating quantitative and qualitative inheritance

QTL with large effects on plant height, anthesis, and silk emergence were detected in this population. Many of these QTL coincide with regions, or loci, defined by alleles with qualitative effects; i.e., mutants. Of the 5 plant height QTL 4 seem to correspond with regions defined by loci such as *an1*, *br1*, *rd1*, and *D8* on 1L; *d5* on 2S; *st1* on 4S; and *Py1* and *rd2* on 6L. Beavis et al. (1991) and Edwards et al. (1992) also observed associations between loci defined by plant height mutants and QTL. Likewise, a QTL for anthesis could correspond to the *id1* locus on 1L; however, it is difficult to distinguish between the possible direct relationship between the QTL and the *id1* locus from the effects of other linked loci affecting plant height.

These observations are in good agreement with a proposed relationship linking quantitative and qualitative genetic variation (Robertson 1985). Briefly, Robertson has suggested that alleles with either quantitative or qualitative effects should be expected to reside at the same locus. Empirical data supporting this hypothesis have been reported in *Drosophila* (Mackay and Langley 1990). The high coincidence between plant height QTL and plant height mutants in maize would seem to support this as well. However, the plethora of mutants defining loci that affect plant height in maize provide ample opportunity for false positive comparisons for this trait. For traits with fewer corresponding mutant loci, such as anthesis and resistance to first-brood European corn borer (1ECB), QTL have been placed to regions previously identified by qualitative mutants for these traits – *id1* and *bx1*, respectively (Lee and Veldboom 1993). In these instances, the potential of false positives is presumably much less than for plant height. For example, *bx1* is, to our knowledge, the only locus in maize with alleles having qualitative effects on host plant resistance to 1ECB.

The identification of QTL in regions lacking loci defined by alleles with qualitative effects suggests an important benefit of this approach to gene mapping and another important link between quantitative and qualitative genetics. Most of the mutants of maize have been detected on the basis of easily recognized effects on readily assessed traits (e.g., color and texture of kernels, gross deviations of plant morphology, development, and pigmentation). These loci have been fortuitously defined through the recovery of alleles with qualitative and, typically, nonlethal effects. Certainly other loci remain to be identified, perhaps through other means. For example, large-scale mapping efforts of cDNA clones should define many new loci. Without extensive sequencing, however, it would be difficult to assign biological significance to many clones, especially if their genetic locations are devoid of loci defined by mutant phenotypes. QTL mapping may, therefore, represent an efficient, albeit crude, genome-wide assessment of regions and their functional significance for various traits.

Although QTL mapping certainly provides less precise genetic information than mutants with qualitative effects, it may offer some advantages because the method does not rely upon the identification of alleles with qualitative effects to define a locus. Moreover, QTL mapping may contribute genetic insight into traits and processes that may not be readily defined by mutants (e.g., resistance to abiotic stress, stalk strength, or resistance to insects). For example, QTL analysis for resistance to 1ECB has revealed a region on 1S with effects nearly equal to those of the QTL on 4S. The QTL on 1S does not have a corresponding mutant locus as does the QTL on 4S with *bx1* (Lee and Veldboom 1993). Indeed, QTL mapping as currently conducted will neither provide the necessary genetic resolution nor contribute definitive fundamental biological information. Nevertheless, the approach should efficiently provide important genetic and functional clues regarding regions to target for more rigorous analysis.

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