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Comparative mapping in $F_{2:3}$ and $F_{6:7}$ generations of quantitative trait loci for grain yield and yield components in maize

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Abstract This study was conducted to compare maize quantitative trait loci (QTL) detection for grain yield and yield components in $F_{2:3}$ and $F_{6:7}$ recombinant inbred (RI) lines from the same population. One hundred and eighty-six $F_{6:7}$ RIs from a Mo17×H99 population were grown in a replicated field experiment and analyzed at 101 loci detected by restriction fragment length polymorphisms (RFLPs). Single-factor analysis of variance was conducted for each locus-trait combination to identify QTL. For grain yield, 6 QTL were detected accounting for 22% of the phenotypic variation. A total of 63 QTL were identified for the seven grain yield components with alleles from both parents contributing to increased trait values. Several genetic regions were associated with more than one trait, indicating possible linked and/or pleiotropic effects. In a comparison with 150 $F_{2:3}$ lines from the same population, the same genetic regions and parental effects were detected across generations despite being evaluated under diverse environmental conditions. Some of the QTL detected in the $F_{2:3}$ seem to be dissected into multiple, linked QTL in the $F_{6:7}$ generation, indicating better genetic resolution for QTL detection with RIs. Also, genetic effects at QTL are smaller in the $F_{6:7}$ generation for all traits.

Key words *Zea mays* · RFLPs · Plant breeding · Genetics · Recombination

Abbreviations *RFLPs* Restriction fragment length polymorphisms · *QTL* quantitative trait loci · *RIs* recombinant inbreds

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Introduction

DNA markers have allowed researchers to investigate the underlying genetic factors controlling quantitative trait variation. With the use of maize RFLP maps, quantitative trait loci (QTL) have been identified and characterized for several traits including plant height (Beavis et al. 1991), low phosphorus stress tolerance (Reiter et al. 1991), Northern corn leaf blight (*Exserohilum turcicum*) resistance (Freymark et al. 1993), gray leaf spot (*Cercospora zeae-maydis*) resistance (Bubeck et al. 1993), European corn borer (*Ostrinia nubilalis* Hubner) resistance (Schön et al. 1993), morphological differences distinguishing maize from teosinte (Doebley et al. 1990), and kernel oil concentration (Goldman et al. 1994).

Grain yield is the most important trait in hybrid maize production, but its evaluation and improvement are difficult because of low heritability (Hallauer and Miranda 1988) and a complex biological basis. Previous studies using molecular markers have improved our understanding of some of the genetic factors controlling grain yield and its components. Isozyme markers and RFLPs have been used to detect QTL controlling several traits, including yield and its components in single-cross F_2 populations. In these instances, trait data were collected from F_2 plants (Edwards et al. 1987; Stuber et al. 1987; Abler et al. 1991; Edwards et al. 1992), $F_{2:3}$ inbred lines (Veldboom and Lee 1994), or backcross lines (Stuber et al. 1992).

RI populations have several advantages for use in mapping QTL, which have been described by several authors (Burr et al. 1988; Cowen et al. 1988; Lander and Botstein 1989; Knapp and Bridges 1990). In maize, RIs have been used to identify QTL for thermotolerance (Frova and Gorla 1993; Sari-Gorla et al. 1992), pollen competitive ability (Ottaviano et al. 1991), and morphological traits (Austin 1995). RIs represent a permanent mapping population in which the expected ratio of parental genotypes is 1:1. This increased replication of homozygous parental genotypes results in an increased power for testing differences

between genotypic classes. The reduced genetic variation within lines results in a greater precision of trait measurements compared to other types of replicated, segregating progeny. QTL with large effects should be detected consistently across generations, but the increased precision of the RIs should allow the detection of QTL with smaller effects. Also, the additional opportunities for recombination should improve the genetic resolution of linked QTL and distinguish linked effects from pleiotropic ones in some instances.

In the study decribed here, grain yield and seven grain yield components were investigated in a population of 186 $F_{6:7}$ RIs derived from a cross between elite inbreds Mo17 and H99. The first objective of our study was to locate and characterize genetic factors associated with trait variation. The second objective was to compare the detection of QTL in the $F_{6:7}$ generation with a previous study using $F_{2:3}$ lines of the same population grown at the same location but evaluated in a different year (Veldboom and Lee 1994). Previous studies have evaluated these traits but have not evaluated the same population at early (F_2-derived) and late ($F_{6:7}$ RIs) stages of inbreeding.

Materials and methods

Formation of the Mo17×H99 mapping population has been described by Veldboom et al. (1994). One hundred and eighty-six unselected $F_{6:7}$ lines were developed with the identity of each F_2 plant being maintained during inbreeding. These include the 147 of the 150 lines analyzed at the $F_{2:3}$ generation.

The 186 RI lines and five entries each of Mo17 and H99 were evaluated in two replications of a 14×14 lattice of single-row plots at the Agronomy and Agricultural Research Station near Ames, Iowa in 1993. Rows were 5.5 m long and spaced 0.76 m apart. Plots were machine-planted on May 15, 1993 at a density of 76,540 kernels per hectare and thinned to 57,400 plants per hectare (24 plants/plot) at the six- to eight-leaf stage. Field plot management practices were similar to those utilized in maize production in the region. The growing season (May through September) had above average rainfall (+652 mm) and below average temperatures (–104 GDD°C). During the pollination period of July and August, the location received 493 mm precipitation above average for this site.

Methods for the collection of trait data were in accordance with Veldboom and Lee (1994). Grain yield and yield components were measured on a plot basis as follows: grain yield (GY) is the total weight (g) of shelled grain converted to Mg ha^{-1}; kernel weight (KWT) is the weight (g) of a 300-kernel sample taken from shelled grain of the plot's total; ear number per plant (ENP) is the total number of ears harvested from the plot divided by the number of plants in the plot; ear length (EL) is the average length (cm) of ten primary (top) ears; ear diameter (ED) is the average diameter (cm) of ten primary ears; cob diameter (CD) is the average diameter (cm) of ten cobs from primary ears; kernel depth (KD) is the calculated depth (cm) from (ED-CD)/2; kernel row number (KR) is the average number of kernel rows of ten primary ears. Plots were harvested by hand.

Genomic DNA isolations and RFLP assays were previously described by Veldboom et al. (1994). Eighty-seven loci were common with the previous study, and additional probes were added to give better coverage for a total of 101 loci from 93 RFLP probes and one morphological marker. $F_{6:7}$ lines were scored as being homozygous Mo17 type (A), homozygous H99 type (B), or heterozygous (H).

Data analysis

The statistical analysis of field data was performed as described by Veldboom et al. (1994). Adjusted entry means were obtained by correcting for lattice effects according to Cochran and Cox (1957). Simple phenotypic correlations between all traits were estimated using the adjusted entry means.Phenotypic correlations between the $F_{2:3}$ and $F_{6:7}$ generations were estimated for all traits using the adjusted entry means from 147 lines evaluated in both generations.

Chi-square analysis was performed at each locus to test for fit to expected segregation ratios. For codominant markers, the expected ratio of A:H:B for 186 F_6-derived lines is 90:6:90. For the four dominant markers, a ratio of 96:90 was used. For linkage analysis, the dominant markers were considered to be scored as only A and B with the realization that, on average, 6 of the lines would be true heterozygotes at the locus. The genomic composition of the $F_{6:7}$ lines was determined by calculating the proportion of loci in each of the genotypic classes (Veldboom et al. 1994).

A linkage map of 101 loci was generated with MAPMAKER/EXP 3.0 (Lander et al. 1987) using the "ri self" setting. The recombination fraction observed in the RI lines (R) is related to the proportion of recombinants in a single meiosis (r) by the equation r=R/(2–2R), where r is the map distance in Morgans (Haldane and Waddington 1931). Linkage was declared to be significant when a LOD threshold of 3.0 and a maximum recombination fraction (r) of 0.4 were met. Genetic distances between markers were estimated by using the Haldane cM function (Haldane 1919). The placement of the centromeres was an approximate based on previous maps (Coe et al. 1990; Veldboom et al. 1994; Matz et al. 1994). On the basis of centromere placement, chromosomal regions will be referred to in this paper as a number followed by L (long) or S (short).

QTL detection

Single-factor analyses of variance were conducted for all pair-wise marker loci and quantitative trait combinations (Edwards et al. 1987). Trait data consisted of adjusted entry means from the lattice design. *F*-tests were used to determine if significant variation in trait expression was associated with the differences in marker-locus genotypic classes. A significant contrast between the homozygous parental genotypic classes was interpreted as evidence for linkage between a QTL and a marker locus. This method is similar to that used by Anderson et al. (1993) except that lines heterozygous for a locus were included herein rather than recorded as missing data. For this study, a significance level of 5% was used as evidence of linkage. The probability of proclaiming a false positive (Type-I error) during any single test was 5%; however, the probability of at least one false positive over the entire genome was 99.4%. Because this is the first RI study in this population, the relaxed rate was used to enable comparisons with previous studies and to reduce the chances of committing a Type-II error. For those loci where the contrast between the homozygous classes was significant, additive effects were calculated according to Edwards et al. (1987). From the total number of loci with a significant contrast, a subset of loci was selected to represent all of the QTL detected for each trait. This was accomplished by selecting the locus with the highest significance from each cluster of closely linked loci with significant contrasts for that trait. If loci on the same chromosome were significant when evaluated simultaneously in a model, they were included in the subset of significant regions as different QTL explaining unique portions of the trait variation. Loci included in the final models are underlined in Fig. 1.

Multiple regression (PROC GLM and PROC REG, SAS) analysis was conducted to estimate the proportion of the phenotypic variation explained by the additive effects. To facilitate regression, we coded the genotypic classes (A, H, B) according to the number of H99 alleles (0, 1, 2). The coefficient of determination (R^2) from the multiple regression estimates the total proportion of phenotypic variation due to the sum of the additive effects. Herein, heritability (h^2) represents an estimate of the proportion of phenotypic variation attributable to genetic sources. The R^2 value was divided by the h^2 to calculate the proportion of the genetic variation due to additive effects for loci linked to all QTL for each trait.

Results

Linkage map and segregation analysis

The RFLP data were used to construct a 101-loci RFLP linkage map (Fig. 1) of 1,408 cM and ten linkage groups. The average distance between adjacent loci was 15.4 cM. With the exception of chromosome arms 7S and 10S, most regions of the maize genome were represented. All loci were placed to linkage groups using the MAPMAKER group command except for npi235, which was placed to chromosome 6 on the basis of previous maps. The total map distances for the F_2 and RI maps using only the 87 marker loci in common are nearly identical at 1,420 and 1,419 cM, respectively (data not shown). Locus order is identical except for 2 loci on the end of the long arm of chromosome 9 separated by 2 cM in the RI map. The loci, npi209 and bnl4.28, are present in opposite order in the F_2 map.

Of the 101 loci, 19 showed significant distorted segregation ratios at probability levels of 0.05 (13), 0.01 (3), or 0.001 (3). Significant deviations were in the form of excess Mo17 homozygotes (5), excess H99 homozygotes (1), excess H99 homozygotes and heterozygotes (12), and excess heterozygotes with a deficiency of both homozygotes (1). Ten of these loci are located in four clusters on chromosomes 1, 4, and 7. Because the deviations occur in both directions (excess of H99 or Mo17 alleles), the overall allele frequencies do not deviate from expectations with 49.5% Mo17 alleles and 50.5% H99 alleles (based on 97 codominant loci). Analysis of the average genomic composition of the $F_{6:7}$ lines indicates no evidence of selection during inbreeding. On average, each line was composed of 47.5% Mo17 type, 48.5% H99 type, and 4.0% heterozygous (calculated with 97 codominant loci). The standard deviations were 9.9%, 9.6%, and 3.0%, which places the genotypic class mean values within the expectations for $F_{6:7}$ lines. The genomic composition among lines ranged from 19.6% to 77.3% homozygous Mo17, 19.6% to 76.3% homozygous H99, and 0.0% to 22.7% heterozygous.

Field trait data

Wilks-Statistic values (in parentheses) indicated normal distributions for GY(0.98), EL (0.99), ED (0.98), CD (0.99), KD (0.98), and KR (0.98). Significant deviations from normality were observed for KWT (0.97) and ENP (0.91). The coefficient of skewness for KWT was 0.35, indicating a skewness toward greater values. For ENP, the coefficient of skewness was –1.37, indicating a skewness toward lesser values.

Genotypic variance components were significant for all traits indicating genetic variability in the population (Table 1). Heritabilities were greater than 0.66 for all traits except ENP, which had a h^2 of 0.48. The h^2 estimates are probably overestimates of the true values because the genotype-by-environment component could not be partitioned from the genotypic variance.

Table 1 Means, ranges, variance components, and heritabilities of grain yield and yield components for maize $F_{6:7}$ lines of Mo17×H99

	GY (Mg ha^{-1})[a]	ENP	EL (cm)	ED (cm)	CD (cm)	KD (cm)	KR	KWT (g 300^{-1})
Means								
Mo17	1.82	0.85	13.9	3.27	2.13	0.57	10.4	62.1
H99	1.66	0.93	12.4	3.32	2.31	0.51	11.4	50.2
$F_{6:7}$ lines	1.41	0.82	12.0	3.26	2.22	0.52	10.9	54.1
Range[b]								
$F_{6:7}$ lines	(0.15–2.66)	(0.38–1.07)	(8.16–16.09)	(2.37–3.84)	(1.73–2.84)	(0.29–0.75)	(8.6–14.0)	(34.5–82.2)
Variance components ($F_{6:7}$ lines)[c]								
σ_g^2±SE	0.19 ± 0.03**	0.011 ± 0.003**	1.57 ± 0.22**	0.044 ± 0.007**	0.025 ± 0.004**	0.0052 ± 0.0009**	0.71 ± 0.09**	69.6 ± 8.3***
σ_e^2	0.15	0.023	0.98	0.034	0.015	0.0053	0.37	19.9
heritability ($F_{6:7}$ lines)								
h^2	0.71	0.48	0.76	0.72	0.77	0.66	0.79	0.87
90% C.I. on h^2	(0.63–0.78)	(0.34–0.60)	(0.69–0.81)	(0.64–0.78)	(0.70–0.82)	(0.57–0.74)	(0.74–0.84)	(0.84–0.90)

[a] See Materials and methods for explanation of abbreviations
[b] Range values do not include RI 137 (yellow-striped, dwarf phenotype) for grain yield (0.00 Mg ha^{-1}), ear number (0.07), and ear length (6.92 cm)
[c] Significance of variance components at 0.01 (**) and 0.001 (***) levels noted

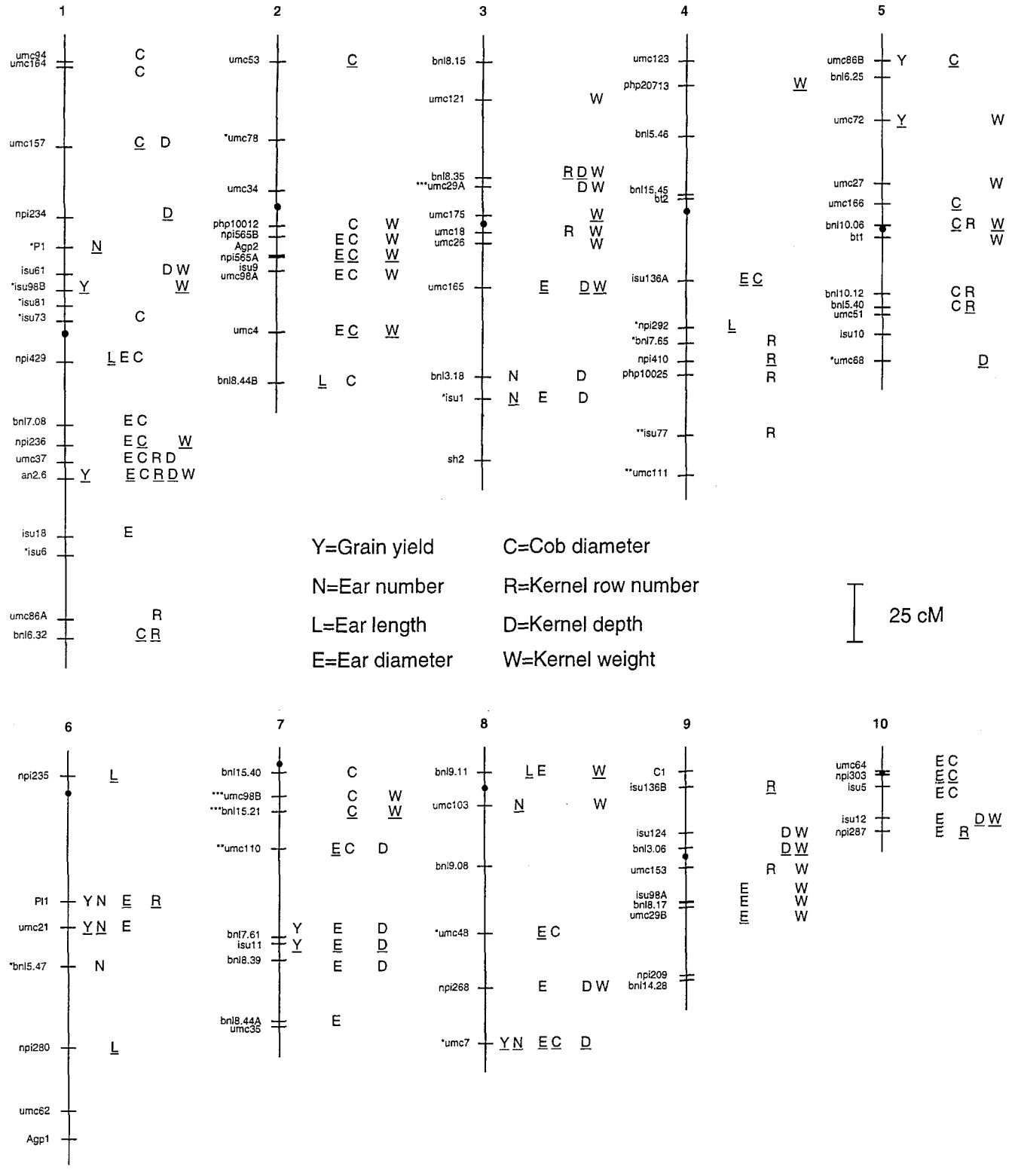

Fig. 1 **Panel A** RFLP marker linkage map of Mo17×H99 $F_{6:7}$ lines (chromosomes 1–5). Loci with significant distortions from the expected segregation are marked (* =0.05, ** =0.01, *** =0.001). All loci significantly ($P \leq 0.05$) associated with grain yield and yield components are labeled with *letters* to the *right* of the chromosome. For each trait, *underlined letters* signify QTL locations. **Panel B** As above, for chromosomes 6–10

The parental checks, Mo17 and H99, had significantly different trait values for KWT, EL, CD, and KR but were not significantly different for GY, KD, ED, and ENP. Mo17 had greater values for GY, EL, KD, and KWT, whereas H99 had greater values for ENP, ED, CD, and KR (Table 1). Transgressive segregation exceeding the high and low parent values was observed for all traits. The RI population means were near the mid-parental values for KWT,

CD, KD, and KR. For GY, ENP, EL, and ED, the RI population mean was below the value of the low parent.

GY had positive significant ($r_p \leq 0.01$) correlations with all yield components except KWT. GY had the highest correlations with ENP (0.70), EL (0.53), ED (0.49), and KD (0.41). All except five of the correlations between the seven yield components were significant ($r_p \leq 0.01$) and positive, with the highest correlations between CD and ED (0.71), KD and ED (0.67), KR and ED (0.43), and between EL and ENP (0.35). The only significant negative correlation was between KWT and KR (–0.22).

QTL analysis

All ten chromosomes were associated with variation in GY and yield components. Of the 808 trait-locus combinations tested, 163 (20%) had effects significant at $P \leq 0.05$. Of the significant loci, 83 (51%) were significant at $P \leq 0.01$ and 33 (20%) were significant at $P \leq 0.001$. Minimum percentages of variation explained by the additive effects of significant loci ranged from 2.1% for ENP and EL to 2.5% for GY, ED, CD, and KWT (Table 2). Also, many regions were associated with several traits, indicating possible linked and/or pleiotropic effects.

Nine loci on five chromosomes were significantly associated with variation for GY (Fig. 1). The linkage between some loci and the results from multiple regression indicated the 9 loci represent 6 QTL accounting for 2.5% to 7.6% of the phenotypic variation (Table 2). Collectively, 6 QTL explained 21.8% of the phenotypic and 30.7% of the genotypic variation. Only 2 of the QTL have alleles from Mo17 (the parent with greater grain yield in this and other environments) contributing to increased GY, but they had the largest (umc7 on 8L) and third largest (isu11 on 7L) effects. The region on 8L had the largest R^2 value, explaining 7.6% of the phenotypic variation. QTL were also detected at umc7 for ED, CD, and KD, with Mo17 alleles contributing to increased trait values. QTL for at least two yield component traits were detected in each region containing a GY QTL.

A total of 63 QTL were identified for the seven yield components (Table 2). ENP with 5 QTL and EL with 6 had the fewest genetic regions associated with trait variation. Eight to twelve QTL were identified for the other five traits. The total amount of phenotypic variation explained for a trait ranged from 17.8% (23.4% of genotypic variation) for EL to 52.7% (68.4% of genotypic variation) for CD. The amount of phenotypic variation explained by individual QTL for yield components ranged from 2.1% to 16.9%. Of the 63 QTL for yield components, 35 (56%) had Mo17 alleles contributing to increased trait values. For KWT, ED, CD, and KR, the QTL explaining the largest portion of the phenotypic variation were associated with Mo17 alleles, whereas the QTL with the largest effects for ENP, EL, and KD were associated with H99 alleles. This was surprising since H99 has lower values for EL and KD (Table 1).

All regions of the map contained QTL for at least one trait, although the distribution of QTL varied considerably among regions. For example, large regions of chromosome arms 2S and 4S were nearly devoid of QTL. In contrast, most regions were associated with more than one trait. The region on 1L near an2.6 was associated with variation for all of the traits except ENP and EL. All of the QTL on 1L near an2.6 had H99 alleles contributing to increased trait values even though the Mo17 parent had higher values for GY, KD, and KWT. The region on 8L near umc7 had QTL for GY, ED, CD, and KD with Mo17 alleles increasing trait values. QTL for five traits were detected on 1S near isu98B. Here, QTL for GY, ENP, and CD were associated with Mo17 alleles, whereas QTL for KWT and KR were associated with H99 alleles. The region of 10L was associated with trait variation for KWT, EL, ED, CD, KD, and KR. Mo17 alleles increased KWT, ED, CD, and KD, whereas H99 alleles increased KR values. On 6L, umc21 was asso-

Table 2 Level of significance, percentage of variation explained, and estimated additive effects of genetic regions significantly associated with yield and yield component variation in the Mo17×H99 $F_{6:7}$ population

Chromosome/ locus[a]	% Variation[b]	Additive effect[c]
Grain yield (Mg Ha^{-1})		
1S/isu98B	2.5	0.080H*
1L/an2.6	3.5	0.095H*
5S/umc72	3.0	0.087M*
6L/umc21	6.2	0.128H***
7L/isu11	3.9	0.100H**
8L/umc7	7.6	0.144M***
Total[d]	21.8 (30.7)	
Ear number $plant^{-1}$		
1S/P1	2.4	0.022M*
3L/isu1	3.5	0.029H*
6L/umc21	10.0	0.048H***
8L/umc103	2.1	0.022H*
8L/umc7	3.6	0.029M**
Total	23.4 (48.8)	
Ear length (cm)		
1L/npi429	3.2	0.26H*
2L/bnl8.44B	2.1	0.21H*
4L/npi292	5.6	0.35M**
6S/npi235	3.3	0.27M*
6L/npi280	2.5	0.23M*
8S/bnl9.11	2.2	0.21M*
Total	17.8 (23.4)	
Kernel row number		
1L/an2.6	2.4	0.15H*
1L/bnl6.32	4.0	0.19M**
3S/bnl8.35	3.1	0.17H*
4L/npi410	7.3	0.26M***
5L/bnl5.40	4.1	0.19H**
6L/P11	2.7	0.16H*
9S/isu136B	2.8	0.16H*
10L/npi287	2.7	0.16H*
Total	22.8 (28.9)	

Table 2 Continued

Chromosome/ locus[a]	% Variation[b]	Additive effect[c]
Kernel weight (g 300^{-1})		
1S/isu98B	3.7	1.77H**
1L/npi236	3.4	1.68H*
2L/Agp2	5.2	2.08M**
2L/umc4	4.6	1.94M**
3S/umc175	4.2	1.88M**
3L/umc165	4.9	2.03M**
4S/php20713	2.8	1.50H*
5S/bnl10.06	3.8	1.78M**
7L/bnl15.21	2.9	1.59H*
8S/bnl9.11	7.8	2.52M***
9S/bnl3.06	5.6	2.15M**
10L/isu12	2.5	1.42M*
Total	39.0 (45.5)	

[a] Chromosome number and arm noted as short arm (S) or long arm (L)
[b] Percentage of phenotypic variation explained by additive effect
[c] Additive effects are followed by M(Mo17) or H(H99) to indicate parental allele contributing to increased trait value. Significance of effects noted with $*P \leq 0.05$, $**P \leq 0.01$, and $***P \leq 0.001$
[d] Total percentage of phenotypic variation explained by multiple regression model. The number in parenthesis indicates percentage of genotypic variation explained. Data for ear diameter, cob diameter, and kernel depth are available upon request

ciated with GY, ENP, ED, and KR with H99 alleles affecting higher trait values for each trait. A QTL was also detected on 6L for EL at npi280 (53 cM distal to umc21) with Mo17 alleles increasing the trait value. QTL associated with Mo17 alleles were detected on 3L near umc165 for KWT, ED, and KD. A QTL associated with H99 alleles was detected for ENP on 3L at isu1 (49 cM distal to umc165). Seven additional regions including 2L(2 regions), 3S, 7L(2 regions), 5S, and 9S contained QTL for two to three traits each.

Comparison of QTL detection in $F_{2:3}$ and $F_{6:7}$ generations

Table 3 presents a comparison of QTL detected using 150 $F_{2:3}$ lines from the same population (Veldboom and Lee 1994) with those detected in this study. The $F_{2:3}$ and $F_{6:7}$ generations were both grown at the same location, but the environmental conditions were different in 1989 and 1993. Conditions during the 1989 growing season (May through September) were the seventh driest on record (–102 mm precipitation) with low soil moisture reserves after a drought in 1988, while GDD accumulation was near average (–7 GDD °C). In contrast, 1993 was one of the wettest on record (+652 mm), and GDD accumulation was below average (–104 GDD °C). The performance of the parental inbreds in the 2 years was very different. In 1989, Mo17 and H99 had GY values of 4.37 Mg ha^{-1} and 1.76 Mg ha^{-1}, respectively. In contrast, yields from the 1993 experiment were 1.82 Mg ha^{-1} for Mo17 and 1.66 Mg ha^{-1} for H99. The methods used for detecting QTL differed in the two studies. Interval mapping was used with the $F_{2:3}$ generation, and single-factor ANOVA was used with the $F_{6:7}$. Studies have reported that the two methods effectively detect the same genetic regions associated with trait variation (Stuber et al. 1992; Bubeck et al. 1993; Pereira and Lee 1995).

Phenotypic correlations between the $F_{2:3}$ and $F_{6:7}$ were significant ($P \leq 0.01$) for KWT (0.39), EL (0.28), ED (0.46), CD (0.53), KD (0.35), and KR (0.36); correlations for GY (0.10) and ENP (-0.03) were not significant. In general, traits with lower h^2 (in the $F_{6:7}$) had lower correlations between generations. This was true for ENP, which had a low h^2 (0.48), but not for GY, which had a high h^2 (0.71).

For GY, a single QTL on 6L was detected in the $F_{2:3}$. This QTL explained 35% of the phenotypic variation, and higher grain yield was associated with the Mo17 alleles. With the $F_{6:7}$ lines, a QTL was also detected on 6L 44 cM from the RFLP locus that defined the $F_{2:3}$ QTL. This QTL explained 6.2% of the phenotypic variation (second largest effect) and was associated with the H99 alleles. With respect to the yield components, 34 QTL were detected in the $F_{2:3}$ generation; in the $F_{6:7}$ lines QTL were detected for the respective traits in 24 (71%) of those regions (Table 3). For all but 2 of the 24 QTL in common, the direction of the parental effects were the same. QTL for ENP were detected in both generations on 6L, but at loci linked by 18 cM with different parental effects. QTL for ED on 6L also differed in parental effect and location by 61 cM. Of the 24 QTL common to both generations, 12 differed in genetic distance by less than 10 cM between the $F_{2:3}$ and $F_{6:7}$ generations. QTL for ED and CD had the closest agreement between the generations. These two traits also had the highest phenotypic correlations between the $F_{2:3}$ and $F_{6:7}$. For ED, all 6 $F_{2:3}$ QTL were detected in the same regions in the $F_{6:7}$ lines; the QTL with the largest additive effect in the $F_{2:3}$, umc37 on 1L, also had the largest effect in the $F_{6:7}$. For CD, all 6 $F_{2:3}$ QTL were also detected in the same regions in the $F_{6:7}$; the QTL with the largest additive effect in the $F_{2:3}$, however, had the fourth largest effect in the $F_{6:7}$. QTL for KR had the least agreement between the two generations with only 1 of 4 $F_{2:3}$ QTL being detected in the $F_{6:7}$ as well as the same parental effect (Mo17). The KR QTL common to both generations on 4L had the second largest effect in the $F_{2:3}$ and the largest effect in the $F_{6:7}$. For ENP, EL, ED, CD, KD, and KWT, the QTL with the largest additive effect in the $F_{2:3}$ was also detected in the $F_{6:7}$ with the same parental contributions. Similar results were observed in this population for plant height and flowering traits (Austin 1995). Of the 23 QTL detected for these traits in the $F_{2:3}$, 16 (70%) were also detected with $F_{6:7}$ lines in the same regions with the same parental effects.

Table 3 Comparison of positions and effects of QTL detected in $F_{2:3}$and $F_{6:7}$ generations of the Mo17×H99 population

Trait[b]	$F_{2:3}$ generation[a]		$F_{6:7}$ generation		Distance[f]
	Chromosome region/locus[c]	Additive effect[d]	RFLP locus[e]	Additive effect	
GY	6L/npi280	1.27M	umc21	0.13H	44
ENP	3L/umc165A	0.12H	isu1	0.03H	39
ENP	6L/bnl5.47	0.15M	umc21	0.05H	18
EL	1S/npi234	0.77M	–[g]		
EL	3L/umc165A	0.12H	–		
EL	5S/umc27	0.59M	–		
EL	6L/npi280	1.39M	npi280	0.23M	0
EL	8L/bnl9.08	0.85M	bnl9.11(8S)	0.21M	32
ED	1L/umc37	0.18H	an2.6[h]	0.10H	7
ED	2L/umc4	0.16M	Agp2	0.07M	34
ED	3L/umc165A	0.12M	umc165A	0.06M	0
ED	6L/npi280	0.16M	Pl1	0.04H	61
ED	7L/umc110	0.15H	umc110	0.06H	0
ED	8L/umc48	0.14M	umc48	0.05M	0
CD	1S/npi234	0.06M	umc157	0.06M	27
CD	1L/bnl7.08	0.11H	npi236	0.06H	9
CD	2L/umc98	0.14M	Agp2	0.06M	8
CD	4L/npi292	0.11H	isu136A[h]	0.05H	21
CD	5L/Bt1	0.07H	umc166(5S)	0.03H	28
CD	7L/bnl15.21	0.10H	bnl15.21	0.04H	0
KR	1S/bnl5.62	0.05H	–		
KR	2S/umc78	0.67M	–		
KR	4S/Bt2	0.29H	–		
KR	4L/umc15[i]	0.45M	npi410	0.26M	3
KD	1S/umc157	0.04H	npi234	0.03H	27
KD	3L/umc165A	0.06M	umc165A	0.02M	0
KD	6L/npi280	0.06M	–		
KD	7L/bnl8.44A	0.02H	isu11	0.03H	31
KD	8L/umc48	0.04M	umc7	0.02M	66
KWT	1L/npi236	3.6H	npi236	1.7H	0
KWT	3S/umc175	7.3M	umc175	1.9M	0
KWT	4L/npi410	5.1H	–		
KWT	5S/umc166	4.7M	bnl10.06	1.8M	15
KWT	6L/npi280	6.0M	–		
KWT	8L/npi268	3.5M	–		

[a] All QTL detected in the $F_{2:3}$ generation are listed and the corresponding region if detected in the $F_{6:7}$ generation
[b] For explanation of abbreviations see Materials and methods
[c] Chromosome number and location noted as short arm (S) or long arm (L)
[d] Addititive effects are followed by M(Mo17) or H(H99) to indicate the parental allele contributing to the increased trait value
[e] RFLP locus explaining the largest portion of variation in a chromosomal region is listed
[f] Distances are from the RFLP locus nearest to the maximum LOD in the $F_{2:3}$ study to the corresponding locus in $F_{6:7}$ study. Distances are based on F_2 map
[g] Regions which were detected in $F_{2:3}$ study but not in $F_{6:7}$ study are noted with "–"
[h] Loci which were mapped in $F_{6:7}$ study but were not mapped in $F_{2:3}$ study. Distances are based on F_6 map
[i] Loci which were mapped in $F_{2:3}$ study but were not mapped in $F_{6:7}$ study

Discussion

QTL detection

Under the 1993 growing conditions, inbreds Mo17 and H99 did not significantly differ in GY. However, significant genetic variation was observed among the RIs, and GY was highly heritable. The GY QTL on 8L represents a difference of 0.29 Mg ha^{-1} between homozygotes at umc7. This 8L region has also been associated with ENP, CD, KD, anthesis, and silk emergence (Austin 1995). Here, Mo17 alleles were associated with increased grain yield traits, whereas H99 alleles delayed flowering. It is possible that 1 QTL could be located near umc7 controlling both yield and flowering in this environment. The QTL from H99 which delays flowering could negatively affect yield in the 1993 environment such that the Mo17 alleles appear to be grain yield QTL. Alternatively, 2 linked QTL are present with opposite additive effects. QTL for yield components and morphological traits (Austin 1995) were detected at loci on chromosome 8 proximal to umc7, and the pattern of their linkage indicates it is likely that linked QTL are in this region, each controlling one or more traits. QTL for

EL, ENP, KWT, anthesis, and anthesis-to-silk interval are all located near the centromeric region defined by bnl9.11-umc103, whereas, QTL for ear-to-tassel length (umc103), ED (umc48), and plant height (npi268) are located at loci distal to this region but proximal to umc7.

At 4 of the 6 GY QTL, H99 alleles increased the trait value. This may be due partially to the stability, or consistency, of GY for that inbred under the wet 1993 and dry 1989 conditions. Although yields were lower for both inbreds in 1993, Mo17 GY was reduced by 42%, whereas H99 GY yield decreased by only 1%. This could be due to the cool, wet conditions of 1993, which delayed anthesis. Significant negative phenotypic correlations were observed between GY and anthesis (–0.45) and between GY and silk emergence (–0.63). Because H99 had a significantly earlier flowering date than Mo17 in 1993, the reduction in GY for Mo17 could be attributed to delayed flowering and an abbreviated grain-fill period. This could result in regions associated with H99 conferring earlier flowering dates and greater GY (1S, 1L, 6L).

Although direct comparisons are hindered by differences in mapping populations (parents and type of progeny) as well as a paucity of common loci and environments, other reports have identified some of the same regions detected in the present study to be associated with GY and related traits. Edwards et al. (1992) detected GY QTL on regions 1S, 1L, 6L, and 8L that were also associated with GY in the present study. GY QTL were detected by Stuber et al. (1992) and in the present study on regions 1L, 5S, 6L, 7L, and 8L. Region 1L, which has been associated with many traits in this population, has been reported to contain QTL for GY in 13 of 18 populations analyzed by Stuber et al. (1992). In a study of traits related to GY or its components, QTL with the largest effects for five of eight traits related to inflorescence structure in a teosinte maize population were also detected on 1L (Doebley and Stec 1991).

QTL associated with several traits

Of the 80 loci significantly associated with trait variation, 53 (66%) were associated with two or more traits. The method of measurement used for some traits could result in such associations (e.g., ED and CD), but others may reflect genetic linkage, pleiotropy, or a consequence of patterns of plant growth and development. Genetic explanations for multiple trait associations include QTL with pleiotropic effects or the presence of linked QTL controlling different traits. The 14.8-cM region on 1L spanning loci npi236, umc37, and an2.6 was observed to be associated with QTL for GY, ED, CD, KR, and KD. Also, QTL explaining the largest proportion of phenotypic variation for plant height, ear height, anthesis, and silk emergence were also detected in this region (Veldboom et al. 1994; Austin 1995). Here, H99 alleles increased values for the yield traits, while Mo17 alleles increased values for plant height and flowering. A single QTL could be present in this region with pleiotropic effects over all these traits with opposite additive effects for the two sets of traits. Another possibility is that 2 or more linked QTL are present controlling the traits.

Previous studies in maize (Abler et al. 1991; Edwards et al. 1992; Veldboom et al. 1994; Veldboom and Lee 1994; Austin 1995) have shown that correlated traits are often associated with common QTL. GY was significantly correlated with all of the yield components except for KWT and had the highest correlation with ENP (0.70). Of the 6 QTL detected for GY and the 5 detected for ENP, 3 were in the same regions for both traits. QTL for at least two yield components were detected in each region containing a GY QTL. Many more QTL were detected for yield components than for GY itself. Each may have an effect on GY, but they may be too small to be distinguished from experimental error in this type of study.

Comparison of QTL detected in $F_{2:3}$ and $F_{6:7}$ generations

RI populations have additional recombination between linked loci and an increased power for detecting QTL (Cowen 1988; Knapp and Bridges 1990). The additional recombination should allow the resolution of some single QTL (in the $F_{2:3}$) into multiple, linked QTL, each with smaller effects. Also, the increased power of the RI population, afforded by the increase in homozygosity, should allow the detection of more QTL with smaller effects than could be detected in the $F_{2:3}$. One possible explanation for QTL being detected in the $F_{2:3}$ but not the $F_{6:7}$ is overdominant gene action. QTL detected in the $F_{2:3}$ with overdominant gene action would not be expected to be detected if the additive effects were small. Of the 20 $F_{2:3}$ yield component QTL with additive, partial dominant, or dominant gene action, 15 (75%) were detected in the same regions with the same parental effects with RIs. Of the 14 $F_{2:3}$ yield component QTL with overdominant gene action, only 7 (50%) were detected in the same regions with the same parental effects.

Overall, more QTL were detected for yield and yield components with $F_{6:7}$ than with $F_{2:3}$ progeny (35 $F_{2:3}$ versus 69 $F_{6:7}$) despite the $F_{2:3}$ being grown in a more favorable environment with greater h^2 values. For grain yield, a total of 6 QTL were detected with $F_{6:7}$ progeny, whereas only 1 QTL was detected with $F_{2:3}$ progeny. For the seven yield component traits, 63 QTL were detected in the $F_{6:7}$, whereas 34 $F_{2:3}$ QTL were detected (Tables 2 and 3). For all of the traits, the QTL additive effects were smaller in the $F_{6:7}$.

For GY, a single major QTL was detected in the $F_{2:3}$ explaining 35% of the phenotypic variation, while all 6 $F_{6:7}$ QTL explained only 21.8% of the phenotypic variation. QTL were detected in both studies on 6L but at different locations with opposite parental effects. The observations suggest that the region on 6L has a major effect on GY, a conclusion that is in agreement with previous reports (Edwards et al. 1992; Stuber et al. 1992). The presence of the QTL in the two generations at loci 44 cM apart with opposite parental effects may indicate the presence of 2 environment-specific QTL. On the basis of the relative per-

formance of the parental inbreds in the two different environments, it seems very plausible that a Mo17 QTL would be detected in the 1989 environment while a H99 QTL would be detected in 1993. Another possibility is a Type-I error in one or both of the generations, but this seems unlikely due to the magnitude of the QTL effects and the importance of this region for GY variation, which has been observed in other studies.

In the $F_{2:3}$ generation, the smallest portion of the phenotypic variation explained by QTL for yield components was 6.2%. Of the 63 QTL detected for the yield components in the $F_{6:7}$ generation, 48 (76%) explained less than 6.2%. These could be QTL with small effects that could not be detected with $F_{2:3}$ progeny. For each of the yield components considered individually, more QTL were detected in the $F_{6:7}$ than in the $F_{2:3}$. For all of the yield components except EL, the amount of variation explained by multiple models including all QTL was greater in the $F_{6:7}$ generation. One factor contributing to the increased variation explained by QTL in the $F_{6:7}$ generation is the additional variation from QTL not detected in the $F_{2:3}$. Although the total variation explained by all of the QTL detected increased for each yield component (except EL), individual QTL for all traits had smaller effects in the $F_{6:7}$.

In several instances, single QTL detected in the $F_{2:3}$ generation appear to be resolved into linked QTL in the $F_{6:7}$ generation. Possible examples may include ED and KWT in this study. For ED, a significant region spanning most of 7L was attributed to a single QTL in the $F_{2:3}$. Inspection of the LOD distribution of the region reveals a large peak at umc110, which corresponds to the location of an $F_{6:7}$ QTL. Two smaller distal peaks (in the $F_{2:3}$) are located near the $F_{6:7}$ QTL at isu11. The single QTL in the $F_{2:3}$ near umc110 explained 15.5% of the phenotypic variation with an additive effect of 0.15 cm. The combined additive effect of the 2 $F_{6:7}$ QTL linked by 42 cM was 0.11 cm and accounted for 11.6% of the phenotypic variation (from the multiple regression model). For KWT, a single QTL was detected in the $F_{2:3}$ generation on chromosome 3 near umc175 with an additive effect of 7.3 g. Two $F_{6:7}$ QTL for KWT were detected on chromosome 3 with one also at umc175 and the second at umc165, which corresponds to a second peak in the $F_{2:3}$ LOD distribution. The single $F_{2:3}$ QTL accounted for 21.9% of the phenotypic variation, while the combined effect of the 2 $F_{6:7}$ QTL linked by 32 cM accounted for 7.0%. Additional examples of 2 linked $F_{6:7}$ QTL being detected in a region with a single $F_{2:3}$ QTL include 8L for ED and 2L for CD. The resolution of multiple, linked QTL was also observed for plant height and flowering traits in this population (Austin 1995). For example, a large region spanning 1L and a portion of 1S was significantly associated with plant height in the $F_{2:3}$and attributed to a single QTL. The region was resolved into 3 QTL in the $F_{6:7}$ with non-significant loci intervening. These observations suggest that the additional recombination experienced during the production of the RIs was an important factor influencing the perception and characterization of QTL.

Implications for marker-assisted breeding

Estimations of QTL number, position, and effects may be biased when assessments are obtained with small sample sizes of populations at maximum linkage disequilibria. An important source of bias is the limited number of recombinants evaluated in such situations. Simulation studies (Gimelfarb and Lande 1994; Edwards and Page 1994) of marker-assisted selection (MAS) have indicated that the efficiency of MAS would decline in later generations partly because of recombination between markers and QTL. Our results support predictions from simulations and further suggest another reason; some of the QTL detected in early generations of maximum linkage disequilibria are indeed due to multiple, linked genes that may be separated via recombination, thus dissipating the effects and reducing the potential gain from selection.

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