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ORIGINAL ARTICLE

Direct mapping of density response in a population of B73 × Mo17 recombinant inbred lines of maize (*Zea Mays* L.)

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Maize yield per unit area has dramatically increased over time as have plant population densities, but the genetic basis for plant response to density is unknown as is its stability over environments. To elucidate the genetic basis of plant response to density in maize, we mapped QTL for plant density-related traits in a population of 186 recombinant inbred lines (RILs) derived from the cross of inbred lines B73 and Mo17. All RILs were evaluated for growth, development, and yield traits at moderate (50 000 plants per hectare) and high (100 000 plants per hectare) plant densities. The results show that genetic control of the traits evaluated is multigenic in their response to density. Five of the seven loci significant for final height showed statistical evidence for epistatic interactions. Other traits such as days to anthesis, anthesis-to-silking interval, barrenness, ears per plant, and yield per plant all showed statistical evidence for an epistatic interaction. Locus by density interactions are of critical importance for anthesis-to-silking interval, barrenness, and ears per plant. A second independent experiment to examine the stability of QTL for barrenness in a new environment clearly showed that the multilocus QTL were stable across environments in their differential response to density. In this verification experiment, the four-locus QTL was used to choose lines with the four unfavorable alleles and compare them with the lines with four favorable alleles and the effect was confirmed.

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Introduction

Genetic variation for complex traits, such as yield potential in elite maize populations, is controlled by many genetic factors, each with relatively small effects (Schön *et al.*, 2004; Holland, 2007). Therefore, the usage of QTL mapping and marker-assisted selection approaches for yield potential in maize is questionable (Holland, 2004). However, yield is a composite trait that is influenced by many stress-related traits. Therefore, it should be possible to dissect maize responses to specific biotic and abiotic stresses into less complex component traits. These component traits may be controlled by smaller sets of genes, making them more amenable to QTL mapping and marker-assisted selection for particular traits (Ribaut *et al.*, 2001; Tuberosa and Salvi, 2004; Hammer *et al.*, 2006; Cooper *et al.*, 2009). They may also be influenced by fewer context-dependent factors. For example, stress resistance traits such as tolerance to inter-plant competition, pests, and drought are suitable

targets to enhance yield stability across environments and production systems.

Research on yield potential and stress resistance in maize, combined with extensive phenotypic selection, has helped achieve significant genetic gains in rainfed yield of maize hybrids (Derieux *et al.*, 1987; Russell, 1991; Tollenaar, 1991; Eyherabide *et al.*, 1994; Sangoi *et al.*, 2002; Duvick, 2005). Genetic yield gain as a result of adaptation to continual increases in plant density and drought stress is perhaps the most evident and quantifiable change in maize hybrids over the years (Edmeades *et al.*, 2006; Lee and Tollenaar, 2007; Messina, 2009). Cardwell (1982) estimated that increased plant densities accounted for 21% of the gain in maize yield in Minnesota (USA), from the 1930s through the 1970s. High plant density increases the deleterious effects of various classes of abiotic and biotic stresses and so increases the need for genetic improvements in stress tolerance (Troyer, 1996). Therefore, increases in plant density were accompanied by the introduction of maize genotypes that could withstand higher densities and achieve higher yields per unit area. For example, several examinations of US hybrids showed that open pollinated cultivars and old hybrids provided their highest yields at the lower densities typical of their era, whereas the newest hybrids yielded most at the higher densities typical of recent years (Russell, 1991; Duvick *et al.*, 2004). Hybrids usually

gave the highest yield when grown at the density for which they were bred. A 1930–1991 time series of 36 hybrids and one open pollinated cultivar for Iowa (Duvick, 1997) showed the same general trends; however, the newest hybrids in this series made only a very small gain in yield when planted at the highest plant density (79 000 plants per hectare) compared with their performance at the intermediate density (54 000 plants hectare). This suggests the possibility that future yield gains from breeding for adaptation to higher plant densities will come at a slower pace or will require more breeding effort. Similar results have been shown for late-maturing hybrids in France and Ontario, Canada (Derieux et al., 1987; Tollenaar et al., 1994). Interestingly, Duvick (1997) also showed that yields of the 1930–1991 series of hybrids did not significantly increase over decades when the hybrids were planted at an extremely low plant density of 10 000 plants per hectare. In this stress-free environment, all hybrids were able to express maximum yield potential per plant. Moreover, under these conditions, the older hybrids showed virtually as much yield potential per plant as the newer hybrids.

Adaptation to higher plant densities has been accompanied by changes in phenological and morphological traits. Anthesis-silking interval (ASI) became shorter with more recent decade of release in three experiments comparing maize cultivars adapted to the US Corn Belt (Meghji et al., 1984; Russell, 1985; Duvick et al., 2004), and this trend was greater in trials grown at higher plant densities (Duvick et al., 2004). Another trait directly associated with response to density is ears per plant. Both total and harvestable ears per plant increased over the decades in a set of Iowa single cross-hybrids representing the decades of the 1930s through the 1970s (Crosbie, 1982). A 1930–2001 time series of 51 hybrids and four open pollinated cultivars adapted to central Iowa showed a highly significant trend toward more ears per 100 plants ($+3.6$ ears per decade) (Duvick et al., 2004). In these two experiments, ears per plant were expressed as means of three densities, in which the medium and high densities were higher than optimum for the older hybrids, and, therefore, were more likely to cause barrenness in those hybrids. Thus, a trend toward more ears per 100 plants (that is reduced barrenness) in the newer hybrids was even greater at the higher densities. Although density is a primary factor in yield potential, QTL mapping for density stress response has been explored in only small samples of near isogenic lines (B73 with Tx 303 introgressions) (Gonzalo et al., 2006).

Earlier studies to map QTL for grain yield under specific abiotic stresses compared positions of grain yield QTL with those of QTL for component traits known to have a function in stress tolerances. Such studies have provided insights into the genetic architecture of plant response to nitrogen deficiency (Bertin and Gallais, 2001; Hirel et al., 2001), water deficiency (Landi et al., 1995; Ribaut et al., 1996; Tuberosa et al., 1998; Sanguineti et al., 1999; Sari-Gorla et al., 1999; Tuberosa et al., 2002; Li et al., 2003), low phosphorus (Reiter et al., 1991), and cold temperature growing conditions (Hund et al., 2004). Although these studies clearly showed an interaction between QTL and stress, they identified QTL for response to stress indirectly by mapping the trait of interest (that is yield) under two or more conditions and overlaying the resulting scans on the common map, to

identify shared and distinct QTL. The QTL is identified in one condition, but not the others are then inferred to be responsible for the plant response to the stress.

To identify genetic factors responsible for response to plant density as measured by ASI, barrenness, and yield per plant, a population of recombinant inbred lines (RILs) derived from the cross B73 × Mo17 was used to map QTL. A direct test of QTL by density interaction was used to identify QTL responsible for the response (Gonzalo et al., 2006). Epistatic effects were strong and multilocus effects were validated for the barreness phenotype in an independent yield trial experiment. Ten RILs representing the predicted favorable allelic combination for the four-locus QTL genotype were tested against 14 RILs representing the predicted unfavorable allelic combination, and the differential response in barrenness to density in these two groups was confirmed. As the validation took place in the subsequent year, this also indicates that these genetic interactions are robust to environmental differences within the same climate zone.

Materials and methods

Maize genotypes
Parental inbreds B73 and Mo17, and 186 B73 × Mo17 (RILs) were studied. The RIL population was earlier genotyped with 233 restriction fragments length polymorphism, single repeat, and isozyme markers (Senior et al., 1996). The two parents were earlier observed to exhibit differences for some of the phenotypes typically associated with density response. Mo17 reaches anthesis earlier and has a longer ASI relative to B73, which sheds pollen later, but has a shorter ASI. Mo17 tends to have higher percentage of barrenness than B73. Genotypes adapted to high density usually exhibit reduced barrenness and shorter ASIs compared with other genotypes at high density (Duvick, 2005).

Experimental design
Entries were randomized into three sets to control for environmental variation. A set within replication design (Hallauer and Miranda, 1988) was used to randomize sets within each of two complete replication blocks at each of four locations (West Lafayette, Wanatah, Romney, IN, USA, and Clayton, NC, USA) in 2003. At each location, a split-split-plot experimental design was used. The whole-plot treatment factor was set (with three levels), the sub-plot factor was density (with two levels), and the sub-sub-plot factor was genotype (with 64 levels per set). Density treatments were a high population density, planted at 100 000 seeds per hectare (38 seeds per row), and a low population density, planted at 50 000 seeds per hectare (19 seeds per row). To account for soil variability, genotypes within a set were planted according to an 8 by 8 lattice design, considering each combination of replication and density as a replication of the alpha design. Field spacing was roughly, but not exactly the same in the NC location. To assure uniform pollen availability, pollinator rows of different maturity were uniformly distributed across the study (Figure 1).

Clayton plots were planted on 23 April 2003, Wanatah plots on 29 April 2003, and West Lafayette and Romney plots were planted on 27 May 2003. The soil type of the
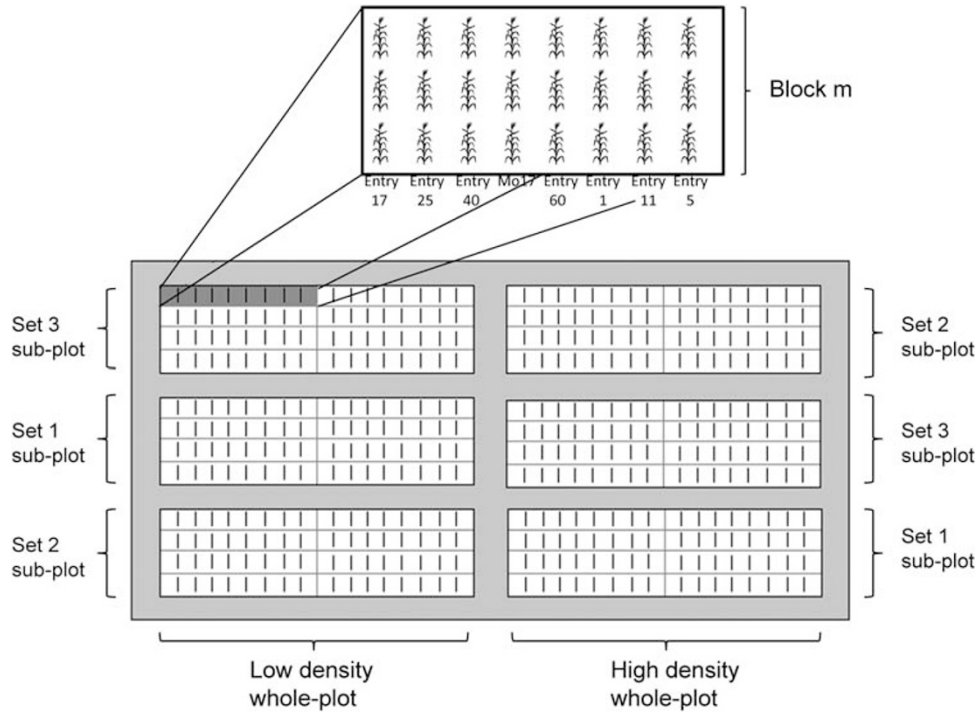
Figure 1 The split-split-plot experimental design. Gray borders represent pollinator rows. Each replicate consists of two whole-plots with the densities. Each whole-plot includes three sub-plots with the sets (each set consisted of 64 entries). The entries within a set were randomized after an 8 (blocks) by 8 (entries) lattice design.

plots at the Purdue University Agronomy Center for Research and Education near Lafayette is Chalmers silty clay loam (fine-silty, mixed, super active, mesic: Typic Endoaquolls), at the Throckmorton-Purdue Agricultural Center near Romney is a Raub silt loam soil (fine-silty, mixed, superactive, mesic: AquicArgiudolls), at the Pinney-Purdue Agricultural Center near Wanatah is Sebewa loam (fine-loamy over sandy or sandy-skeletal, mixed, super active, mesic: Typic Argiaquolls), and the Central Crops Research Station (Clayton, NC, USA) is Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults).

Data collection
Although all plants within an RIL are genetically nearly identical, multiple plants per row were measured to reduce the effect of any micro-environmental influences within the plots. Days after planting to 50% anthesis and silking were recorded on each plot as the date when 50% of all the plants in a plot were observed to have the first visible anther or the first visible silk, respectively. ASI was determined by subtracting days to anthesis from silking. Plant height for five consecutive plants per row from the ground to the collar of the flag leaf was measured 2 weeks after anthesis. The first plant measured was randomly chosen within a row, excluding the first plant and the last plant to avoid border effects. Proportion of barren plants was determined by dividing the number of plants with no ears by the total number of plants in a row. The primary ear of five randomly chosen plants that were not barren were collected, dried to constant weight, and weighed. All data are available on request and included as Supplementary material.

Data analysis
The median of the five heights scored on each plot was used in the analysis. The mean of the five ear weights was used in the analysis. Days to anthesis data were strongly skewed. Several survival models were evaluated to determine the probability distribution that best fit the data. As censoring for days to anthesis was <3%, and log-normal survival models fit well, we log-transformed days to anthesis data and used a mixed linear model to analyze the data. This approach is equivalent to fitting a log-normal model in survival time analysis with no censored data (Lawless, 1982; Vermerris and McIntyre 1999).

Mixed-effects linear models were fitted for each trait separately. The initial model used was

$$
\begin{aligned}
y_{ijklmno} ={} & \mu + L_i + \delta_{ik} + S_l + (LS)_{il} + (\delta S)_{ikl} + D_j \\
& + (LD)_{ij} + (DS)_{jl} + (LD\delta)_{ijk} + (LDS)_{ijl} + (LD\delta S)_{ijkl} \\
& + \beta_{ijklm} + R_{ln} + (LR)_{i\,ln} + (DR)_{j\,ln} + (LDR)_{ij\,ln} + \in_{ijklmno}
\end{aligned}
$$

$$(1)$$

$y_{ijklmno}$ is the value of the trait for the oth plot from the RIL n within set l planted in the density treatment j in block m within replicate k within location i. L_i, δ_{ik}, S_l, D_j, β_{ijklmn}, and R_{ln} represent location, replicate within location, set, density, incomplete block within location by replicate by set by density, and RIL within set effects, respectively, and $\in_{ijklmno}$ represents a random error term. Location, replicate within location, block within location by replicate by set by density, RIL, and their interactions were considered as random effects. Density was considered as a fixed effect. Model described in Equation (1) was reduced by testing $(LDR)_{ij\,ln}$ for each trait. The likelihood ratio test (LRT) and the Wald test indicated

that this effect was not significant for all traits, so the term was removed from the model. Similarly, the overall significance of set and the interaction terms involving set were tested for each trait by analyzing the data as if no set were present using the mixed model

$$
\begin{aligned}
y_{ijklmno} =& \mu + L_i + \delta_{ik} + S_l + (LS)_{il} + (\delta S)_{ikl} + D_j + (LD)_{ij} \\
& + (DS)_{jl} + (LD\delta)_{ijk} + (LDS)_{ijl} + (LD\delta S)_{ijkl} + \beta_{ijklm} \\
& + R_{ln} + (LR)_{i\,ln} + (DR)_{j\,ln} + (LDR)_{ij\,ln} + \in_{ijklmno}
\end{aligned}
$$

(2)

Bayesian Information Criterion (BIC) (Bozdogan, 1987) was used to determine whether model described in Equation (1) could be reduced to model described in Equation (2). In addition, the standard errors to compare two RILs from the same set, one RIL from set 1 and one RIL from set 2, one RIL across densities, and one RIL across two locations were estimated for both models. When BIC for model described in Equation (2) was smaller and the standard errors for the comparisons were virtually unaltered, model described in Equation (2) was used as the overall model.

QTL analysis was performed using a mixed-model approach, which follows from the popular regression-based approaches (that is Haley and Knott, 1992; Jansen, 1992; Jansen and Stam, 1994; Whittaker et al., 1996). We began with a single-marker analysis. This was performed by adding terms representing marker main effect and marker interactions with density and location to the overall model as follows:

$$
\begin{aligned}
y_{ijklmno} =& \mu + L_i + D_j + (LD)_{ij} + M_p + (LM)_{ip} + (DM)_{jp} + (LDM)_{ijp} \\
& + \delta_{ik} + (D\delta)_{ijk} + \beta_{ijkm} + R_{np} + (LR)_{inp} + (DR)_{jnp} + \in_{ijkmno}
\end{aligned}
$$

(3)

where M_p, $(LM)_{ip}$, $(DM)_{jp}$, and $(LDM)_{ijp}$ represent the main effect of the marker, and the interactions marker by density, marker by location, and marker by location by density, respectively. The random effect of RIL R_{np} is nested within marker and represents the residual genetic variance.

To detect epistatic effects, it is necessary to consider a large-model space. In this case, several tactics have been proposed. First, it is necessary to consider at least the set of all possible pairs of loci (Holland, 1998). It is also desirable to consider larger groups of loci, including sets of three and four QTL simultaneously with interaction terms (Carlborg et al., 2000). However, as the number of markers is quite large, the number of possible models quickly becomes large and the computation involved in fitting all such models is formidable. To reduce the computational burden, without losing information in the model space, we used a strategy-based on the underlying genetic map, initially proposed by Coffman et al. (2005) and summarized in Figure 2. Using the model described in Equation (3), we selected the marker with the minimum BIC for each chromosome. For long chromosomes (1 and 5), we selected one marker on each chromosome arm. We also selected two markers per chromosome when the two markers were at $>50\,\mathrm{cM}$ from each other and had similar BIC. In this way, 12–14 markers were selected for each trait in this step.

The second step consisted of fitting all possible multiple-marker models with up to four markers using a cell mean parameterization (that is if 12 markers were

selected, 781 multiple-marker models: 66 two-marker, 220 three-marker, and 495 four-marker models are evaluated). This parameterization is equivalent to the factor-effects parameterization with all possible interaction terms in the model, including interactions with density. This full-model approach has recently been shown to have superior power compared with incremental approaches, which first identify main effects and later fit interaction terms (Marchini et al., 2005). We evaluated models with up to four markers because we expect to have about 11 RILs in each of 16 four-marker genotypic combinations, resulting in reliable estimates of cell means and residual genetic variance $R_{n(p)}$. Models with more than four markers had the limitation that many of the genotypic combinations were missing, and the number of RILs for some combinations were too small to estimate $R_{n(p)}$ precisely. The BIC criterion was used to select a subset of multiple-marker models that best fit the data. On the basis of BIC, more than one multiple-marker model fit the data equally well. All markers included in these multiple-markers models that fit the data equally well were considered for further analysis.

Two procedures were used to determine the final model for each trait. One of the procedures consisted of fitting a model with all marker main effects, all two-way marker by density and marker by marker interactions, and all three-way marker by marker by density interactions for all markers selected in the earlier step (for example if four markers were selected in the earlier step, the model would have four-marker main effects, four marker by density interactions, six marker by marker interactions, and six marker by marker by density interactions). These models were fit using maximum likelihood. This model was then gradually reduced through backward elimination. The full and reduced models were compared using the overall likelihood and constructing an LRT. The criterion to eliminate a term from the model was that the P-value for the LRT (Bozdogan, 1987) was above 0.1. The second selection procedure consisted of fitting all possible two-marker models including the two-marker main effects, the two marker by density interactions, the marker by marker interaction, and the marker by marker by density interaction for the markers selected in the earlier step. For example, if four markers were selected in the earlier step, this selection procedure would consist of fitting six two-marker models, each with its corresponding two-marker main effects, two marker by density interactions, a marker by marker interaction, and a marker by marker by density interaction. All terms that were significant ($P < 0.1$) in at least one of these separate models were included in a final model for this procedure.

These terms obtained from the two selection procedures were then compared. Generally, the two-model selection strategies agreed. In the rare cases in which the final models disagreed, the term that was significant using one of the selection procedures was included in the final model. Finally, a last round of backward elimination was then used to reduce the model. These models were estimated using maximum likelihood. The LRT was used to test whether the full model fit significantly better than the reduced model at a threshold of $\alpha = 0.10$.

Once the final model for each trait was determined, marker main effects and marker by marker, marker by
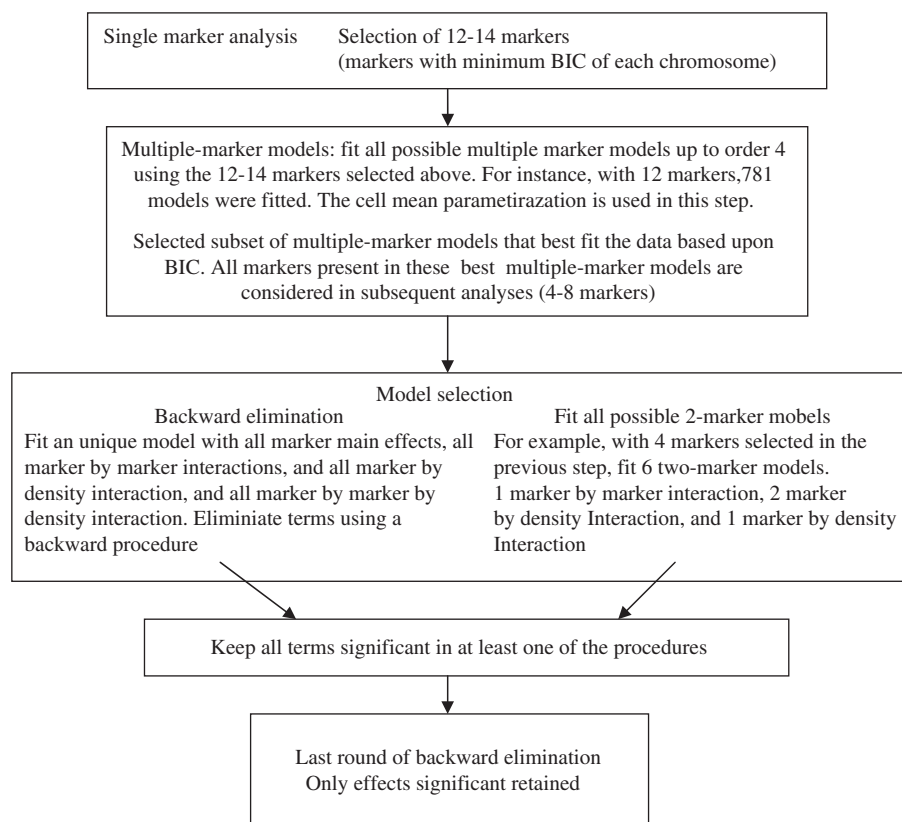
Figure 2 Scheme of the statistical approach used to detect and map QTL.

location, and marker by density interaction effects were tested using the ESTIMATE function of PROC MIXED with Satterthwaite approximation for degrees of freedom. Mixed models were fit using Proc Mixed of SAS Version 9.1.3 (Littel *et al.*, 1996; SAS Institute Inc, 2006). Heritability and its approximate standard error for each trait were estimated for each trait from the selected model and for each density separately after Holland *et al.* (2003). Genotypic correlations and their approximate standard errors were estimated for each pair of traits at each density separately using a multivariate extension of the mixed model in Equation (2) after Holland (2006). Owing to convergence problems with the multivariate model in PROC MIXED, however, the multivariate mixed model was fit and genotypic correlations and their approximate standard errors were estimated with ASReml Version 2.0 software (Gilmour *et al.*, 2003).

Verification of QTL for barrenness

A validation experiment was performed to determine whether the QTL detected for barrenness were reproducible in different environmental conditions. A second objective of the validation experiment was to evaluate the impact of the QTL for barrenness on the yield per unit area (instead of per plant, as it was evaluated in the 2003 season). To evaluate these objectives, a yield trial was planted in three locations in 2005. A total of 24 RILs were selected from the population. These lines were selected on the basis of their marker type for a four-locus QTL model for barrenness. Ten of the lines had the *predicted unfavorable alleles* at all four loci, whereas the

other 14 lines had the *predicted favorable alleles* at all four loci. These contrasting sets were used to obtain an independent verification for the effect of the QTL for barrenness. The inbred line Mo17 was included as a check entry. All seeds were made in the same nursery field in the summer of 2004 at the Purdue University Agricultural Research Center in West Lafayette, Indiana.

Entries were arranged in a split-plot design with three replicates in each of three locations. The whole-plot treatment factor was density with two levels, high density (100 000 seeds per hectare (38 seeds per row)) and low density (50 000 seeds per hectare (19 seeds per row)). The sub-plot treatment factor was the RIL genotype with 25 levels (the 24 RILs and the check line Mo17). To account for soil variability, genotypes within a density were randomized according to a 5 by 5 lattice design. To assure uniform pollen availability, pollinator rows of different maturity were uniformly distributed across the study.

Each sub-plot consisted of four rows measuring 5 m long with 0.76-m inter-row spacing and a 1-m alley at the end of each plot. The two border rows were planted with either inbred lines B73 or Mo17, and the two center rows were planted with the entries. The determination of inbred line for the two border rows was on the basis of the height of the entry. For short RILs, Mo17 was used in the border rows, whereas B73 was used in the border rows when the RIL was tall. This was performed to assure that all the entries in the study were subjected to similar levels of competition.

The experiment was planted on 2 May 2005 at the Pinney-Purdue Agricultural Center near Wanatah,

Indiana, on a soil classified as Sebewa loam, on 4 May 2005 at the Purdue University Agronomy Center for Research and Education near West Lafayette, Indiana, on a soil classified as poorly drained Chalmers silty clay loam, and on 11 May 2005 in the Throckmorton-Purdue Agricultural Center near Romney, Indiana, on a Raub silt loam soil.

Barrenness was scored on all plants per plot in each environment. A plant was considered barren if no ear was visible after physiological maturity. Proportion of barren plants was determined by dividing the number of plants with no ears by the total number of plants in a row. The center two rows of each sub-plot were harvested with a self-propelled combine. Grain weight per plot was corrected to 15.5% grain moisture.

ANOVA models for the split-plot designs were fitted as follows:

$$y_{ijklmn} = \mu + L_i + D_j + (LD)_{ij} + \delta_{ik} + (D\delta)_{ijk} + R_i$$
$$+ (LR)_{il} + (DR)_{jl} + (LDR)_{ijl} + \beta_{ijkm} + \in_{ijlmn}$$

(4)

y_{ijkmn} is the trait value of the nth plot from the RIL l, planted in the density treatment j, block m, replicate k, and location i. L_i, D_j, δ_{ik}, R_l, and B_{ijkm}, represent location, density, replicate within location, RIL, and block within replicate within location by density, respectively, and $\in_{ijkmn}$ is a random error. Location, replicate within location, block within a location by replicate by density, and their interactions were considered random. Density and RIL were considered fixed.

Results

Evaluation of full RIL population
The main effects of density treatment were significant ($P < 0.05$) for ASI, final height, days to anthesis, ears per plant, and barrenness, but not significant for yield per plant ($P = 0.0684$). The average effect of higher density was to increase days to anthesis from 76.9 to 77.4 days after emergence, ASI from 2.31 to 3.03 days, barrenness from 3.3 to 7.4%, and final height from 173 to 184 cm. Higher densities also reduced ears per plant from 0.96 to 0.89 and yield per plant from 82 to 67 g pl^{-1} (grams per plant).

The parental line responses to the density treatment generally followed the trend of the overall means. B73 was 19 and 17 cm taller at the end of the season, reached anthesis 3.2 and 3.4 days later, and had ASIs 2.0 and 2.3 days shorter than Mo17 ($P < 0.05$) at low and high densities, respectively (Table 1). B73 had fewer barren plants (0.04% at low and 0.75% at high), more ears per plant (0.09 at low density and 0.04 at high density), and each ear produced more grain (11.38 g pl^{-1} at low and 14.80 g pl^{-1} at high) than Mo17 (Table 1). Transgressive segregation was observed for all traits in the study (Table 1). Except for low values of barrenness, RIL extremes were significantly different than the parental extremes.

Heritability
Heritability was very similar in both high and low density. Heritability of family means were generally moderately high, with the greatest heritabilities estimated for flowering and plant height traits ($h^2 \geqslant 0.93$) and, except for barrenness traits, all family mean-basis heritability estimates were $\geqslant 0.75$, indicating reliable phenotypic data for QTL mapping (Table 2). Barrenness had lower heritability at low density ($h^2 = 0.49$) because this trait is not highly expressed at lower densities. At high density, the heritability of barrenness increased to 0.79, reflecting its more consistent expression under population density stress.

Genetic correlations
Many significant genetic correlations were observed among the traits measured and they were generally similar in both high and low density (Table 3). ASI, barrenness, ears per plant, and yield per primary ear were highly correlated to each other. ASI was positively associated with barrenness and negatively associated with ears per plant and yield per ear. Increased barrenness was negatively associated with ears per plant and yield per ear, and ears per plant were positively associated with increased yield per ear (Table 3). Final height was associated with days to anthesis.

QTLs in the B73 × Mo17 population
Significant marker by marker and/or marker by marker by density interactions were detected for all traits. Most of the markers in the final model exhibited significant interaction with other markers regardless of the significance of their main effect. Table 3 presents markers for which the marker main effect and/or the marker by density interaction was significant, and includes infor-

Table 1 Parental line means, recombinant inbred line (RIL) population mean, extremes of the RIL population, least significant difference (LSD) for line mean comparisons, for each of six traits measured on 186 RILs at low and high densities

	Final height (cm)		Days to anthesis		ASI		Barrenness		Ear per plant		Yield per ear (g)	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
B73	183.7	192.6	77.8	78.3	0.39	0.90	0.98%	2.80%	1.07	0.96	98	83
Mo17	164.4	175.3	74.6	74.9	2.41	3.21	1.02%	3.55%	0.98	0.94	87	68
RIL mean	176.2	186.0	76.9	77.5	2.27	2.97	3.36%	7.27%	0.96	0.88	81	67
RIL minimum	139.6	149.4	72.1	72.7	−0.13	0.43	0.36%	2.33%	0.61	0.51	35	28
RIL maximum	236.1	244.5	82.0	82.6	6.56	7.74	18.55%	30.73%	1.22	1.05	106	91
LSD 5%	6.2	6.2	0.9	0.9	1.18	1.17	5.34%	5.34%	0.08	0.08	12	12
	6.8	6.7	1.0	0.9	1.23	1.23	5.67%	5.52%	0.09	0.08	15	15

Abbreviation: ASI, anthesis-silking interval.
The standard errors for line mean comparisons varied with the number of times two lines appeared in the same block in the lattice design. Therefore, the minimum and the maximum LSD are given.

mation on significant epistatic interactions with other markers in the model. For example, only two of the eight markers for final height did not show epistatic interaction with any other markers in the model (Table 4). Similar results were observed for the other five traits (one of two for days to anthesis, three of seven for ASI, three of six for barrenness, two of seven for ears per plant, and zero of three for yield per plant) (Table 4). In addition, some markers showed significant epistatic effects and significant marker by density interaction effects without significant main effects. Tables 5–7 show the effects of the markers with significant marker by marker and/or marker by marker by density interactions when the epistatic interaction was taken into account. Note that

some markers that are absent from Table 4 show significant effects only in specific combinations with alleles at other markers. For example, for plant height, the homozygous B73 genotype at umc133b significantly reduced final height only when the homozygous Mo17 genotype was present at the other two loci (Table 5). In addition, this umc133b effect was independent of density when the homozygous Mo17 genotype was present at umc85, but was only significant at low density with the homozygous Mo17 genotype at amp1.

Figure 3 shows the position of the loci significant for each trait. Uncertainty of the map positions of QTLs is illustrated with boxes spanning several centimorgans that include the loci reported in Tables 4–7. The precision of QTL positions can vary within the same study and confidence intervals are not necessarily symmetrically distributed around the marker that most likely associated with the trait. QTL limits were determined by examining neighboring markers. The end of the interval is defined as the first marker that has no significant effect.

Interestingly, five of the seven QTL for barrenness were significant only at high density (Figure 3), indicating a strong QTL by density interaction for this trait. Three of these seven QTL for barrenness overlapped with QTL for ears per plant (QTL on chromosomes 3, 4, and 10), for which increasing barrenness was associated with reduced ears per plant or vice versa. However, QTL for ears per plant on chromosomes 6, 7, 8, and 9 were not associated with QTL for barrenness, suggesting that ears per plant may be governed by additional genetic factors beyond those that influence barrenness. Four of the seven QTL for barrenness mapped to positions that overlap with four QTL for ASI (chromosomes 1, 4, 5, and 10) (Figure 3). In all four cases, alleles that reduced ASI also reduced barrenness. However, the other four QTL for ASI (one of the QTL on chromosome 1, and the QTL on chromosomes 2 and 7) did not map to positions near QTL for barrenness and/or ears per plant (Figure 3). Out of five QTL for yield per plant, only one (on chromosome 3) clearly overlapped with a QTL for barrenness. This was expected as yield per plant was determined on the basis of harvesting plants that had at least one ear and indicates that barrenness and individual plant grain

Table 2 Heritability estimates (and their standard errors) for each phenotype measured

Trait	Heritability (plot mean basis)	Heritability (family mean basis)
Height	0.738 (0.0228)	0.952 (0.00613)
Height (low density)	0.751 (0.230)	0.946 (0.00676)
Height (high density)	0.735 (0.0241)	0.942 (0.00732)
Ears per plant	0.350 (0.326)	0.796 (0.0265)
Ears per plant (low density)	0.341 (0.0358)	0.750 (0.0315)
Ears per plant (high density)	0.411 (0.0351)	0.812 (0.0235)
Yield per plant	0.363 (0.0338)	0.788 (0.0279)
Yield per plant (low density)	0.461 (0.0358)	0.834 (0.0212)
Yield per plant (high density)	0.359 (0.0351)	0.781 (0.0277)
Barrenness	0.240 (0.0321)	0.671 (0.0433)
Barrenness (low density)	0.135 (0.0296)	0.493 (0.0660)
Barrenness (high density)	0.385 (0.0356)	0.792 (0.0263)
ASI	0.367 (0.0314)	0.830 (0.0226)
ASI (low density)	0.369 (0.0340)	0.80 (0.0254)
ASI (high density)	0.405 (0.0347)	0.810 (0.0237)
Anthesis	0.702(0.0245)	0.949 (0.00672)
Anthesis (low density)	0.722 (0.0246)	0.944 (0.007002)
Anthesis (high density)	0.707 (0.0255767)	0.9361838 (0.0079196)

Abbreviation: ASI, anthesis-silking interval.
Estimates are given for the combined data as well as for each density separately. Anthesis is the log of the days to the observation of the first anther.

Table 3 Genetic correlations among traits estimated separately in the two densities

		Genetic correlation high density	Standard error	Genetic correlation low density	Standard error
Ear per plant	Yield per plant	0.6141	0.0639	0.5977	0.0678
Barreness	Yield per plant	−0.3856	0.0843	−0.4838	0.0973
Barreness	Ear per plant	−0.9118	0.0185	−0.8683	0.0546
ASI	Yield per plant	−0.4718	0.0775	−0.4081	0.0828
ASI	Ear per plant	−0.6022	0.0615	−0.5872	0.0755
ASI	Barreness	0.5006	0.0722	0.5288	0.104
Anthesis	Yield per plant	−0.2647	0.0822	−0.3229	0.0761
Anthesis	Ear per plant	−0.1778	0.082	−0.1495	0.0868
Anthesis	Barreness	0.0095	0.0857	0.1557	0.1065
Anthesis	ASI	−0.092	0.0837	−0.1322	0.0887
Height	Yield per plant	0.027	0.0883	−0.0645	0.0843
Height	Ear per plant	0.0547	0.0848	0.1411	0.087
Height	Barreness	−0.1227	0.0851	−0.1311	0.1066
Height	ASI	−0.0696	0.0847	−0.0361	0.0901
Height	Anthesis	0.5356	0.0574	0.4715	0.0618

Abbreviation: ASI, anthesis-silking interval.

Table 4 Effects of replacing B73 alleles by Mo17 alleles on the six traits for markers with significant marker main effects and/or marker by density interactions estimated by fitting the best multiple marker model in the B73 × Mo17 RIL population

Trait	Chromosome	Marker	Density	Effect	Epistatic with
Final height[a]	1	umc133b	Low	−6.16*	amp1(1), umc85(6)
			High	−3.62	
	2	umc53a	Mean	4.75*	amp1(1), umc85(6) nc134(9)
	3	phi036	Low	2.54	umc85(6)
			High	−0.55	
	4	umc52	Low	−1.55	phi069(7), umc89(8)
			High	−3.81	
	7	phi069	Mean	13.79****	umc52(4)
	8	umc89	Mean	14.67****	umc52(4)
	9	nc134	Mean	−17.62****	umc53a(2)
	10	phi035	Mean	−7.74**	
Days to anthesis	2	npi254a	Low	−0.04	
			High	0.29	
	8	bngl666	Mean	0.78*	phi070(6)
ASI		umc76	Mean	0.59**	
	1	amp1	Low	0.24	csu164b(5), umc21(6)
			High	0.65**	
	4	umc49d	Mean	−0.73***	bngl180(2), bnl9.11(8)
	5	csu164b	Mean	0.76**	amp1(1), bngl381(2)
		nrz5	Low	0.41	
			High	0.82***	bngl381(2), phi059(10)
	8	bnl9.11	Mean	−0.81**	umc49d(4)
	10	phi059	Low	−0.55*	bngl381(2), bngl180(2) nrz5(5), umc124(8)
			High	−1.27****	
Barrenness (%)	2	umc49a	Low	0.75	
			High	2.36**	
	3	bngl197	Low	−0.60	
			High	−2.69**	
	4	umc49d	Mean	−2.74***	phi077(6), bngl210(10)
	5	umc156	Mean	2.19*	
	6	phi077	Low	−1.44	umc128(1), umc49d(4)
			High	−2.98***	
	10	bngl210	Low	−0.58	umc49d(4)
			High	−1.98*	
Ears per plant	4	umc49d	Low	0.043**	phi091(7), csu093(9)
			High	0.066****	
	5	bnl5.40	Mean	−0.058***	phi089(6), bngl210(10)
	6	phi089	Mean	−0.030*	bnl5.40(5), phi091(7) bz1(9)
	7	php20581a	Low	0.027	bngl240(8)
			High	−0.001	
		phi091	Low	0.002	umc49d(4), phi089(6)
			High	−0.024	
	9	bz1	Mean	0.035*	phi089(6)
	10	bngl210	Low	0.022	bnl5.40(5)
			High	0.042**	
Yield per plant	2	phi098	Mean	−4.90*	bnl7.65(4)
	3	bnl6.16	Mean	8.55***	umc70c(9)
	4	bnl7.65	Low	14.89****	phi098(2)
			High	8.60***	

Abbreviations: ASI, anthesis-silking interval; RIL, recombinant inbred line.
If the marker by density interaction was significant, the effect of the marker is given for each density. Otherwise, an average effect across densities is reported. *, **, ***, **** Significant at $P \leqslant 0.05$, 0.01, 0.001, or 0.0001, respectively.
[a]The final model includes these 8 main effects and 11 epistatic interactions.

yield may be controlled by different genetic factors (Figure 3).

Validation of QTL for barrenness
A yield trial experiment was performed in 2005 to determine whether the multilocus effects detected for barrenness were reproducible in different environmental conditions. A second objective of the validation experiment was to evaluate the impact of the QTL for barrenness on the yield per unit surface (instead of per plant, as it was evaluated in the 2003 season). A total of 24 RILs was selected from the population on the basis of their genotype for a four-locus QTL model for barrenness

Table 5 Epistatic interactions for plant height

Chromosome	Marker	Density	Marker effect estimate conditional on homozygous genotype at second marker (chromosome)			
			amp1(1)		umc85(6)	
			B73	Mo17	B73	Mo17
1	umc133b	Low	−0.37	−11.94**	0.59	−10.36**
		High	−1.27	−5.96		
			umc133b(1)		umc53a(2)	
			B73	Mo17	B73	Mo17
1	amp1	Low	1.86	−9.72**	−9.61**	0.32
		High	−3.02	−7.71*		
			umc85(6)			
			B73	Mo17		
		Low	−8.41*	0.55		
		High	−8.17*	−2.56		
			amp1(1)		umc85(6)	
			B73	Mo17	B73	Mo17
2	umc53a	Low	−0.22	9.71**	12.67****	−3.18
		High				
			nc134(9)			
			B73	Mo17		
		Low	3.27	5.40		
		High	5.87	4.44		
			umc85(6)			
			B73	Mo17		
3	phi036	Low	−4.43	6.42		
		High				
			phi069(7)		umc89(8)	
			B73	Mo17	B73	Mo17
4	umc52	Low	5.43 4.79	−8.53*	3.11	−8.48**
		High		−12.42***		
			umc133b(1)		amp1(1)	
			B73	Mo17	B73	Mo17
		Low	6.81	−4.14	−3.02	5.93
		High			−1.59	4.02
			umc53a(2)		phi036(3)	
			B73	Mo17	B73	Mo17
6	umc85	Low	9.26**	−6.59	−4.09	6.76
		High				
			umc52(4)			
			B73	Mo17		
7	phi069	Low	21.02****	7.07*		
		High	22.14****	4.92		
			umc52(4)			
			B73	Mo17		
8	umc89	Low	20.46****	8.87**		
		High				
			umc53a(2)			
			B73	Mo17		
9	nc134	Low	−18.96****	−16.83****		
		High	−16.62****	−18.05****		

Effects of replacing B73 alleles by Mo17 alleles estimated by fitting the best multiple marker model in the B73 × Mo17 recombinant inbred line (RIL) population. If the marker by marker by density interaction was significant, the effect of the marker is given for each density. Otherwise, an average effect across densities is reported.
*, **, ***, **** Significant at $P \leqslant 0.05$, 0.01, 0.001, or 0.0001, respectively.

developed on the basis of the 2003 evaluation of the full RIL population. The four key loci controlling barrenness were umc128 on chromosome 1, bngl197 on chromosome 3, umc49d on chromosome 4, and umc156 on chromosome 5. Ten of the lines had the unfavorable alleles (increased barrenness) at all four loci

Table 6 Epistatic interactions for days to anthesis and ASI

Chromosome	Marker	Density	Marker effect estimate conditional on homozygous genotype at second marker (chromosome)			
Days to anthesis						
			Nc131(2)		Bngl279(9)	
			B73	Mo17	B73	Mo17
1	umc133b	Low	−0.23	−1.82**	−1.14**	−0.92*
		High			−1.39***	−0.68
			umc133b(1)			
			B73	Mo17		
2	nc131	Low	0.01	−1.58****		
		High				
			Phil21(8)			
			B73	Mo17		
6	Phi070	Low	0.31	−0.68		
		High	0.38	0.35		
			Phi070(6)			
			B73	Mo17		
8	bngl666	Low	0.67	1.04*		
		High	0.99**	0.95		
			Umc133b(1)			
			B73	Mo17		
9	Bngl279	Low	0.45	0.67		
		High	0.43	1.15**		
ASI						
			csu164b(5)		umc21(6)	
			B73	Mo17	B73	Mo17
1	amp1	Low	0.23	0.26	1.10***	−0.22
		High	0.91*	0.38		
2	bngl381		csu164b(5)		nrz5(5)	
			B73	Mo17	B73	Mo17
		Low	0.14	−0.34	−0.72*	0.38
		High	−0.48	0.03		
			phi059(10)			
			B73	Mo17		
		Low	−0.36	0.16		
		High	−0.15	−0.31		
2	bngl180		umc49d(4)		umc124(8)	
			B73	Mo17	B73	Mo17
		Low	−0.36	−0.24	−1.10**	0.31
		High	−0.14	−0.86**		
			phi059(10)			
			B73	Mo17		
		Low	0.16	−0.96**		
		High				
			bnl180(2)		bml9.11(8)	
			B73	Mo17	B73	Mo17
4	umc89	Low	−0.83*	−0.71	−0.77*	−0.77*
		High	−0.32	−1.04	−0.97**	−0.39
5	nc134		amp1(1)		bngl381(2)	
			B73	Mo17	B73	Mo17
		Low	0.60	0.64	0.86**	0.38
		High	1.17***	0.63	0.64*	1.16**
5	nrz5b		bngl381(2)		phi059(10)	
			B73	Mo17	B73	Mo17
		Low	0.06	1.16***	0.02	1.21***
		High				

Table 6 Continued

Chromosome	Marker	Density	Marker effect estimate conditional on homozygous genotype at second marker (chromosome)			
6	umc21		ampl(1)			
			B73	Mo17		
		Low	0.44	−0.87**		
		High				
8	umc124		bngl180(2)		phi059(10)	
			B73	Mo17	B73	Mo17
		Low	−0.72*	0.69*	−0.48	0.45
		High				
8	bnl9.11		umc49d(4)			
			B73	Mo17		
		Low	−0.81*	−0.81*		
		High	−1.10**	−0.52		
10	phi059		bngl381(2)		bngl180(2)	
			B73	Mo17	B73	Mo17
		Low	−0.81*	−0.29	−0.35	−1.47****
		High	−1.10**	−1.35***		
			nrz5(5)		umc124(8)	
			B73	Mo17	B73	Mo17
		Low	−1.50****	−0.32	−1.38***	−0.44
		High				

Abbreviation: ASI, anthesis-silking interval.
Effects of replacing B73 alleles by Mo17 alleles for markers with significant marker by marker and/or marker by marker by density interactions estimated by fitting the best multiple marker model in the B73 × Mo17 recombinant inbred line (RIL) population. If the marker by marker by density interaction was significant, the effect of the marker is given for each density. Otherwise, an average effect across densities is reported. *, **, ***, ****Significant at $P \leqslant 0.05$, 0.01, 0.001, or 0.0001, respectively.

(homozygous B73, Mo17, Mo17, and B73 alleles at umc128, bngl197, umc49d, and umc156, respectively) (see Tables 4 and 7), whereas the other 14 lines had the favorable alleles (reduced barrenness) at all four loci. This particular four-locus model was selected because of the number and balance of genotypes available in each group compared with other multiple-locus models.

The main effects of density treatment were highly significant ($P < 0.01$) for ears per plant, barrenness, and yield per unit surface. RILs with the unfavorable allelic combination had significantly higher percentage of barrenness and fewer ears per plant than RILs with the favorable allelic combination (Table 8) at both densities. The difference between the two RIL groups was significantly larger ($P < 0.001$ for barrenness and $P < 0.01$ for ears per plant) at high than at low density. RILs with the unfavorable allelic combination had 16.5% higher barrenness and 0.19 fewer ears per plant at low density, but these differences were 23% and 0.24, respectively, at high density (Table 8). RILs with the unfavorable allelic combination yielded significantly less than RILs with the favorable allelic combination (Table 8) at both densities (0.51 T ha^{-1} and 0.60 T ha^{-1} at low and high density, respectively). However, the interaction between allelic combination and density was not significant ($P = 0.4455$). Severe moisture limitations during the pre-anthesis period in the 2005 season in all three locations may have added an additional source of stress on top of the competition for light and water imposed by density treatments. Severe moisture deficits from the V2 to the V15 stage may explain the very low yields observed in the validation experiment.

Discussion

These results show that multiple loci have a function in the genetic control of all the traits studied in the paper, and in the phenotypic response to density. Statistical models indicate that these loci generally interact epistatically. In many ways, this is unsurprising, as epistasis has been earlier reported to have a function in phenotype expression (Avery and Wasserman, 1992). Epistasis has been proposed to have a significant function in some elite maize hybrids (Lamkey et al., 1995; Holland, 2001). Most epistatic interactions observed in this study resulted in a change in magnitude, but not direction of the effect of one QTL across homozygous genotypes at a second QTL (Tables 5–7). However, a few cases of stronger interaction effects were observed, at which the sign of the QTL effect at one locus changed depending on the genotype at a second locus (Tables 5–7). Most dramatically, the effect of replacing a homozygous B73 genotype with a homozygous Mo17 genotype at marker umc124 (chromosome 8) on ASI changed from significantly negative to significantly positive at about the same magnitude across genotypes at bngl180 (chromosome 2; Table 6). This illustrates a case in which both loci are expected to have nearly zero main effects, but strong epistatic interactions.

The significance of epistatic interactions in earlier QTL studies in maize has been generally on the basis of marker by marker interaction, in which the markers has been selected on the basis of a main-effect QTL model (for example Stuber et al., 1992; Melchinger et al., 1998; Lu et al., 2003). This approach implies that only markers with sizable main effects after adjusting for the other

Table 7 Epistatic interactions for barrenness (%), ears per plant, and grain yield per plant

Chromosome	Marker	Density	Marker effect estimate conditional on homozygous genotype at second marker (chromosome)			

Barrenness (%)

			phi077(6)			
			B73	Mo17		
1	umc128	Low	1.13	1.38		
		High	0.37	3.48**		

			phi077(6)		bngl210(10)	
			B73	Mo17	B73	Mo17
4	umc49d	Low	−1.51*	−2.68	−0.88	−4.50****
		High	−1.09	−5.66****		

			umc128(1)		umc49d(4)	
			B73	Mo17	B73	Mo17
6	phi077	Low	−1.57	−1.32	−0.87	−2.03
		High	−4.53***	−1.43	−0.70	−5.27****

			umc49d(4)			
			B73	Mo17		
10	bngl210	Low High	0.56	−3.13**		

Ears per plant

			phi091(7)		csu093(9)	
			B73	Mo17	B73	Mo17
4	umc49d	Low	0.025	0.085***	0.047*	0.040
		High			0.050*	0.083***

			phi089(6)		bngl210(10)	
			B73	Mo17	B73	Mo17
5	bnl5.40	Low	−0.070**	−0.048*	−0.014	−0.101****
		High	−0.092****	−0.022		

			bnl5.40(5)		phi091(7)	
			B73	Mo17	B73	Mo17
6	phi089	Low	−0.041	−0.019	−0.052*	−0.008
		High	−0.066**	0.004	−0.035	−0.027

			bzl(9)			
			B73	Mo17		
		Low High	0.002	−0.063**		

			bngl240(8)			
			B73	Mo17		
7	php20581a	Low High	0.049*	−0.024		

			umc49d(4)		phi089(6)	
			B73	Mo17	B73	Mo17
7	phi091	Low	−0.041	0.019	−0.021	0.024
		High			−0.028	−0.020

			php20581a(7)			
			B73	Mo17		
8	bngl240	Low High	0.050*	−0.023		

			phi089(6)			
			B73	Mo17		
9	bz1	Low High	0.067**	0.003		

			bnl5.40(5)			
			B73	Mo17		
10	bngl210	Low High	0.076***	−0.011		

Yield per plant

			umc70c(9)			
			B73	Mo17		
1	umc107a	Low	6.13	−9.42**		
		High	2.12	−3.06		

Table 7 Continued

Chromosome	Marker	Density	Marker effect estimate conditional on homozygous genotype at second marker (chromosome)			
			bnl7.65(4)			
			B73	Mo17		
2	phi098	Low	−9.29**	−0.91		
		High	−4.15	−5.22		
			umc70c(9)			
			B73	Mo17		
3	bnl6.16	Low High	13.15***	3.94		
			phi098(2)			
			B73	Mo17		
4	bnl7.65	Low	10.70**	19.07****		
		High	9.13**	8.06*		
			umc107a(1)		bnl6.16(3)	
			B73	Mo17	B73	Mo17
9	umc70c	Low	10.53*	−5.02	7.26*	−1.95
		High	5.15	−0.03		

Effects of replacing B73 alleles by Mo17 alleles for markers with significant marker by marker and/or marker by marker by density interactions estimated by fitting the best multiple marker model in the B73 × Mo17 recombinant inbred line (RIL) population. If the marker by marker by density interaction was significant, the effect of the marker is given for each density. Otherwise, an average effect across densities is reported. *, **, ***, **** Significant at $P \leqslant 0.05$, 0.01, 0.001, or 0.0001, respectively.

marker main effects in the model will be tested for epistatic interactions. To overcome this limitation Holland (1998) proposed scanning the genome using pairwise sets of markers, and Carlborg *et al.* (2000) proposed scanning for epistasis using larger numbers of markers. Recent empirical work suggests that approaches, which consider full models, and scan the genome including epistatic terms have greater power than models considering only main effects (Coffman *et al.*, 2005; Marchini *et al.*, 2005; Xu and Jia, 2007; Manichaikul *et al.*, 2009). Here, we begin with the full model, and to ensure that the model space is fully explored, we select a marker for each linkage block on the basis of the BIC. In this approach, a significant effect is not required to include the marker representing this linkage block in more complex models. This approach is similar to the one proposed by Holland (1998) and has been used for QTL mapping in maize before (for example Cardinal *et al.*, 2001; Szalma *et al.*, 2002; Mihaljevic *et al.*, 2005). This procedure can identify unlinked epistatic QTL (Coffman *et al.*, 2005), which could explain the sizable number of significant epistatic interactions detected in our study in comparison to other QTL studies. The significance of epistasis in our models was apparent for all traits. When an LRT was used to determine the overall significance of the epistatic interactions for all six traits evaluated in this study, the tests indicated that the model with epistasis significantly fit the data better ($P < 0.01$) than the reduced model without epistatic interactions.

The importance of the QTL by density interaction is consistent with results from other whole-genome scan studies on plant response to stress such as nitrogen deficiency (Bertin and Gallais, 2001; Hirel *et al.*, 2001), water deficiency (Landi *et al.*, 1995; Ribaut *et al.*, 1996; Tuberosa *et al.*, 1998; Sanguineti *et al.*, 1999; Sari-Gorla *et al.*, 1999; Tuberosa *et al.*, 2002; Li *et al.*, 2003), low

phosphorus (Reiter *et al.*, 1991), and cold temperature growing conditions (Hund *et al.*, 2004). Physiological response to stress in general may be similar as response to density stress and enhanced tolerance to crowding may confer enhanced tolerance to other stresses. In these studies, the presence of QTL by stress level interaction was indirectly inferred from comparing QTL under two or more conditions and overlaying the resulting scans on the common map, to identify shared and distinct QTL. However, such an identification of QTL positions for response to stress is unsatisfactory, as there is no statistical assessment of the interaction between QTL and stress level (Gonzalo *et al.*, 2006). We found numerous significant interactions between QTL and stress level in this limited population in which only two alleles are segregating. Out of the 33 QTL reported in Figure 3, 17 show evidence of differential response to density. Perhaps, the most compelling evidence for QTL by density interaction was observed for barrenness. For this trait, five of the seven loci showed significant effects only at high density.

Adaptation to high density has been responsible for a significant portion of maize yield gains during the last decades (Duvick, 2005). Shorter ASI, reduced barrenness, and more ears per plant at high density are all traits that are strongly associated with adaptation to increase in plant density and resulted from direct or indirect selection. Genotypes adapted to high density do not show increased yield potential per plant, even though they have greatly increased maize yield potential per unit area (Duvick 1997; Sangoi *et al.*, 2002). We have identified QTL, which have significant effects on ASI, barrenness, and ears per plant in a B73 × Mo17 recombinant inbred population. The loci identified here can be compared by map position to loci earlier identified in B73 × Mo17 mapping populations. F4 lines from this cross were studied by Beavis *et al.* (1994). Two of the QTL
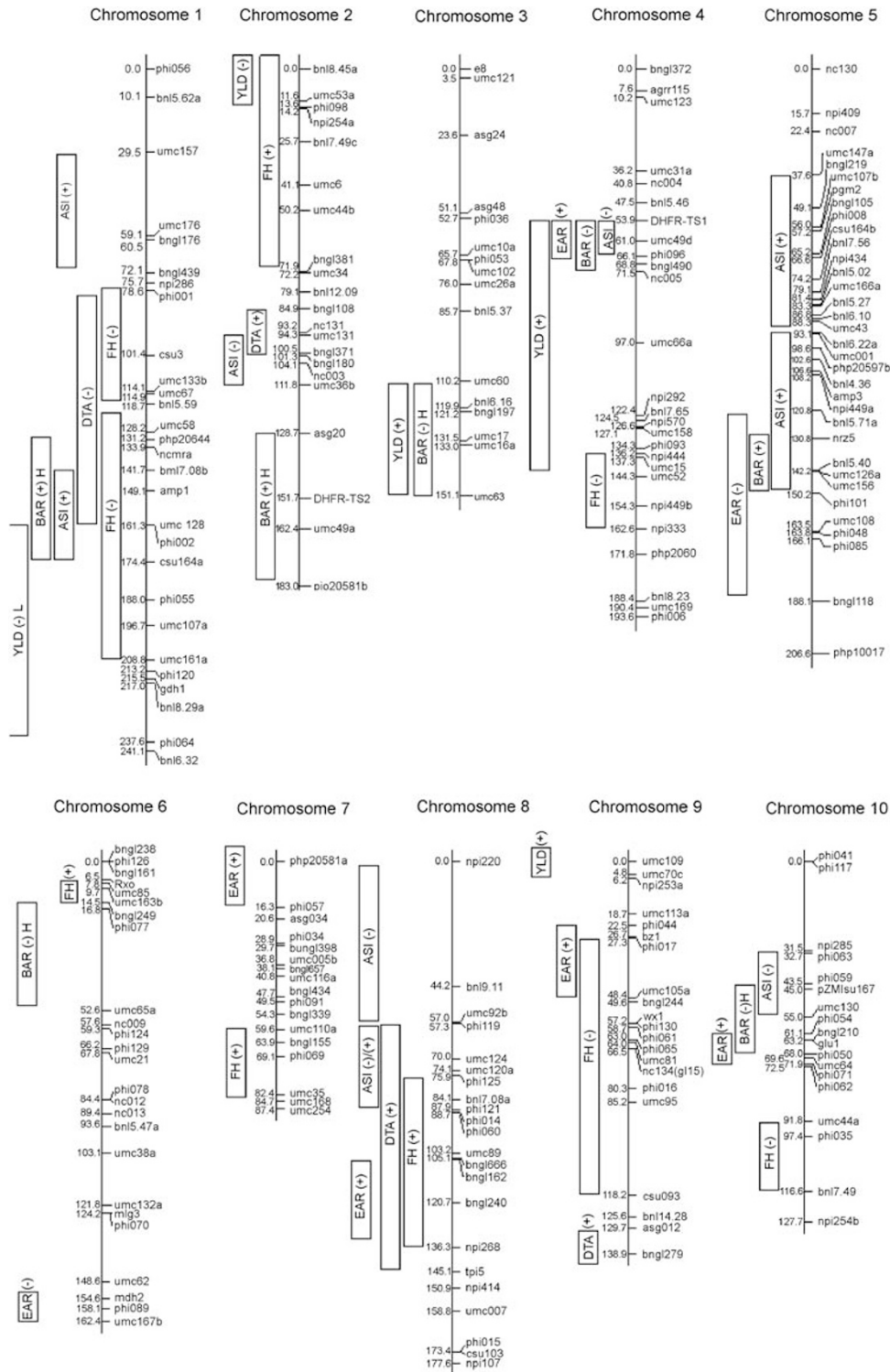
Figure 3 QTL positions for the six traits evaluated in the B73 × Mo17 RIL population. Boxes to the left of linkage groups represent the QTL for the traits indicated in the box: final height (FH), days to anthesis (DTA), ASI, barrenness (BAR), ears per plant (EAR), yield per plant (YLD). The (+) indicates that the B73 allele at this QTL increased the value of the trait, (−) indicates that the B73 allele at this QTL reduced the value of the trait. H and L indicates that the QTL was only significant at low (L) or high (H) density. The marker within each box that was used to represent each box is reported in Tables 3–6.

for heat units to anthesis in Beavis *et al*. (1994) mapped to regions covered by our QTL for days to anthesis on chromosomes 1 and 8, and the effect of the B73 allele at these two QTL were consistent between studies. In addition, Beavis *et al*. (1994) reported five QTL for grain yield (on a per unit surface area basis), three of which mapped to regions associated with barrenness, ear per plant, and yield per plant in our study. The QTL for grain

Table 8 Mo17 means, means of the selected RILs, and least significant difference (LSD) for comparisons between lines with the favorable and unfavorable alleles for each of three traits evaluated in the validation study in 2005 at low and high densities

	Yield (T ha^{-1})		Barrenness		Ears per plant	
	Low density	High density	Low density	High density	Low density	High density
Mo17	2.77	3.33	5.54%	9.22%	0.96	0.91
RIL mean	1.69	1.90	16.05%	28.83%	0.86	0.72
Unfavorable allelic comb.	1.39	1.55	25.67%	42.27%	0.75	0.58
Favorable allelic comb.	1.90	2.15	9.18%	19.23%	0.94	0.82
LSD (5%) favorable vs unfavorable	0.29		4.27%		0.05	

Abbreviation: RIL, recombinant inbred line.

yield on chromosome 1 in Beavis et al. (1994) mapped to a position spanned by the coverage region for the QTL for yield per plant (Figure 3), and in both cases, the B73 allele reduced yield. QTL for grain yield on chromosomes 2 and 9 in Beavis et al. (1994) mapped to regions covered by a QTL for barrenness and a QTL for ears per plant in our study Figure 3. In both studies, the favorable alleles were Mo17 and B73 alleles, respectively. Backcrosses of F3 lines from this cross to either parent were studied by Stuber et al. (1992). Stuber et al. (1992) reported a major yield QTL on chromosome 3, in the same region as our QTL for barrenness and yield per plant, and the B73 allele at this locus conferred a yield increase relative to Mo17 allele. In this study, the B73 allele at this region significantly increased yield per plant and reduced barrenness. Stuber et al. (1992) also reported a major yield QTL on chromosome 10, in the same region as our QTL for barrenness and ears per plant (Figure 3). The B73 allele at this locus conferred a yield increase relative to Mo17 allele in Stuber et al. (1992), and in our study, the B73 allele reduced barrenness and increased ear number per plant. The two QTL for ears per plant detected in the Mo17 backcross in Stuber et al. (1992) mapped around the QTL for ear per plant on chromosomes 7 and 8 in our study. Finally, the barren stalk1 locus, at which mutant alleles can eliminate the production of female inflorescenses (Gallavotti et al., 2007), is located near the major barrenness and yield QTL on chromosome 3, suggesting that this locus may also contribute to natural variation for barrenness in maize. Overlapping QTL in barrenness and grain yield suggest possible genetic mechanisms for carbon allocation as may be expected (Hammer et al., 2009; Messina, 2009).

The combined effect of four of the QTL for barrenness detected in 2003 was verified in an independent large-plot yield trial in a different year, at three locations. RILs with the predicted unfavorable alleles had a significantly higher percentage of barrenness in the yield trial than RILs with the predicted favorable combinations. The result from the initial full population study, in which two of the QTL for barrenness mapped to regions also associated with ears per plant (on chromosomes 4 and 5) led us to also predict that selection on barrenness QTL would also lead to correlated responses in ears per plant. This prediction was borne out in the yield trial study, in which the RILs with the unfavorable alleles had significantly fewer ears per plant than the RIL with the favorable alleles.

In this investigation, we mapped QTL for response to plant density for final height, days to anthesis, ASI,

barrenness, and ear per plant in a population of RILs derived from the cross B73 × Mo17. Our results suggest that QTL by density interactions are of importance when mapping stress-related traits such as barrenness and ear per plant. QTL mapping of these traits accounting for QTL by density interactions seems to work well for these traits, as it was confirmed in a subsequent yield trial experiment. Our results also suggest that epistatic interactions have an important function in the genetic control of the traits evaluated in our study. These results show that the specific abiotic stress of density is a component of yield for which much of the genetic response can be dissected into a small set of four regions of interacting loci. This limited number of regions makes them suitable targets for marker-assisted selection to enhance yield stability across environments and production systems.

Conflict of interest

The authors declare no conflict of interest.

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