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COTTON IMPROVEMENT

Quantitative Trait Loci Associated with Agronomic and Fiber Traits of Upland Cotton

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INTERPRETIVE SUMMARY

Most genetic traits useful for cotton improvement are influenced by several genes. These are called quantitatively inherited traits. Knowledge of the location where these genes reside on the chromosomes would be useful to the cotton breeder, especially if easily measured molecular markers are closely linked or associated with the specific quantitative trait loci (QTLs). We crossed two very different lines of cotton and studied the joint segregation of restriction fragment length polymorphism (RFLP) molecular markers and agronomic and fiber traits. One parent was from the multiadversity breeding program in Texas, MARCABUCAG8US-1-88, and the other parent was a Delta-type commercial cultivar HS 46. By determining the relationships among RFLP markers and agronomic and fiber traits, we showed that several of the RFLP markers are associated closely with specific agronomic and fiber traits. We determined the location of 100 QTLs which mapped to 60 different maximum likelihood locations in 24 linkage groups. Many of these were closely associated with RFLP molecular markers. This information should be of value to breeders as well as provide a beginning basis for cloning specific genes that influence important agronomic and fiber traits. This study also showed that several traits are truly quantitative in nature, that is, controlled by several genes, as they were associated with more than one molecular marker in more than one linkage group.

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Abbreviations: LOD, log to the base 10 of the ratio of the odds of linkage to no linkage; QTL(s), quantitative trait locus (loci); RFLP, restriction fragment length polymorphism.

ABSTRACT

Identification of quantitative trait loci (QTLs) for agronomic and fiber traits in upland cotton (*Gossypium hirsutum* L.) and their allelic association with molecular markers would be useful in cotton breeding. We used the mixed model approach of Zhu and Weir (1998) to analyze for QTLs associated with 19 agronomic and fiber traits across 96 F_2-derived families from the cross of two cotton lines, MARCABUCAG8US-1-88 x 'HS 46' (female parent). In the mixed model, molecular markers are random variables and QTLs are fixed variables. Thus, with the mixed model analysis, the QTLs are not dependent upon a particular fixed set of markers being in the model. The model also provides estimates of additive and dominance genetic effects as well as the direction of the effects of alleles from both parents. The fiber and agronomic traits, except seed index and bloom rate, were measured in F_2-derived F_5 families. We mapped 100 QTLs to 60 maximum likelihood positions in 24 linkage groups. Several QTLs influence more than one trait. The most frequent association of QTLs with multiple traits was for fiber traits related to maturity and fineness. A positive correlation among traits would be beneficial for marker-assisted selection in plant breeding as well as for cloning genes for transformation. For example, in linkage group 14 near markers C117C5RI and F26ERI, a QTL is located that affects micronaire, arealometer high pressure reading, weight fineness, and wall thickness. In linkage group 19, four closely linked QTLs, located in an 8 cM region near marker C80F1RV, influence strength, fineness, and maturity of fiber. Maximum likelihood locations such as those obtained in this study do not necessarily represent physical distances, thus, a physical map of linkage groups is also needed.

The identification and characterization of genes controlling traits of use in plant improvement has long been a focus of scientists in the agricultural community. Recent advances in molecular biological techniques have helped to hasten the realization of these goals. The association of molecular markers with desirable quantitative traits should contribute to the discovery of genetic variability and aid in the selection of desirable parents and progeny. The absence of environmental influence on molecular markers adds to their usefulness in marker-assisted selection for QTLs. The identification of multiple QTLs with varying genetic effects for an individual trait provides evidence of the quantitative nature of the genes influencing the trait. When the QTLs are also closely linked with molecular markers, the opportunity exists for marker-assisted selection for the trait.

The MAPMAKER\ QTL method (Paterson et al., 1988) has been the standard for interval mapping for several years. This method uses a model that considers only two loci at a time for the calculations. The method of composite interval mapping that Zeng (1993, 1994) developed includes marker information for controlling background noise while searching for the QTL. The marker effects, as well as the QTL effects, in this model are treated as fixed effects. Therefore, the estimated QTL effects could be affected by the markers included in the model. Zhu and Weir (1998) proposed a new method that uses a mixed model approach for composite interval mapping of QTLs. In their mixed model, QTLs are fixed variables while molecular markers are random variables. Thus, the estimates of the QTLs will not depend upon a particular fixed set of markers being in the model. The model also provides estimates of additive and dominance effects of QTLs.

Meredith (1992), in a study of heterosis and varietal origins, reported on the first RFLP evaluations in upland cotton, *G. hirsutum* L. Reinisch et al. (1994) developed a detailed RFLP map of cotton with 41 linkage groups by using an interspecific F_2 population from the cross of *G. hirsutum* L. race "palmeri" x *G. barbadense* L. accession K101. Shappley et al. (1996) established five linkage groups in a cross of two upland *G. hirsutum* L. cottons. Shappley et al. (1998) also developed a genetic linkage map with 31 linkage groups in upland cotton from a cross of two *G. hirsutum* L. lines. This map was based on segregation in 96 F_2:F_3 families scored for 129 probe-enzyme combinations that resulted in 138 RFLP loci (120 in linkage groups and 18 nonlinked, Shappley et al., 1998). These were established with an LOD (log to the base 10 of the ratio of the odds of linkage to no linkage) score of greater than 3.0. There were 84 codominant loci of which 76 segregated normally (1:2:1 ratio) for codominant alleles and 54 dominant loci at which only one allele was identified, of which 50 segregated normally (3:1 ratio). These 31 linkage groups covered 865 cM or an estimated 18.6% of the genome (Shappley et al., 1998).

Shappley (1996) provided the first linkage map of QTLs in a cross of upland cotton. However, while carefully examining these data in preparation for writing this manuscript, we discovered a computer coding error in the QTL data of Shappley (1996). Thus, no correct linkage map with QTLs and associated molecular markers has been reported in crosses of two *G. hirsutum* lines. Such maps may be especially valuable for analysis and detection of variability in *G. hirsutum* including elite germplasm. A map showing a QTL for several fiber traits from a cross of *G. hirsutum* x *G. barbadense* was published recently (Jiang et al., 1998). Interspecific incompatibility usually complicates segregation in interspecific hybrids. Upland cultivars (*G. hirsutum*) comprise more than 90% of cotton acreage in the world. Identification of QTLs and their association with molecular markers in segregating generations following crosses of upland cotton is of great interest to cotton breeders. The identification of QTLs controlling traits of interest to breeders of upland cotton and their association with RFLP molecular markers was the focus of this research.

MATERIALS AND METHODS

Material and Traits Analyzed

QTLs affecting 19 agronomic and fiber traits were searched for among the 31 linkage groups established by Shappley (1996) and Shappley et al. (1998) in upland cotton. Molecular methods and mapping methods establishing the 31 linkage groups are given in Shappley et al. (1998). We used

the same cross for the QTL analysis as Shappley (1994, 1996), Shappley et al. (1996), and Shappley et al. (1998) used to establish the RFLP linkage map in upland cotton.

All measurements were made on 96 F_2-derived families from the cross of two *G. hirsutum* L. lines, MARCABUCAG8US-1-88 as male parent x 'HS 46' female parent. These parents are very diverse in agronomic and fiber traits as well as diverse for RFLP markers. A cross was made in 1991, and in 1992 nine F_1 plants were grown and analyzed to determine if restriction fragment length variability was observed among the plants. Some variability was observed, thus one plant was chosen to self-pollinate to produce the F_2 population.

One hundred F_2 seed were planted in the greenhouse in the winter of 1992 and 96 plants grew and were allowed to self-pollinate. This planting was the beginning of successive generations of F_2-derived families. Bulk samples of leaves were collected from F_2:F_3 families and analysis with RFLP probes was procured from Biogenetics Services Incorporated, Brookings, SD. Biogenetic Services Inc. developed the probes using cDNA cotton leaf and fiber libraries. Individual families were self-pollinated and seed bulked by families in the F_3 and F_4. In the spring of 1995 two-row plots of F_5 seed were planted and agronomic and fiber data were collected for the QTLs study.

Conventional and arealometer fiber measurements, as well as selected agronomic measurements, were made in the F_5 generation. Blooming rates and seed indexes were measured in the F_3 and F_4 generations, respectively.

Agronomic and fiber traits are listed in Table 1. Samples for lint percentage measurements, and all measurements of fiber traits were made from hand-picked boll samples, ginned on a 10 saw gin, at Mississippi State, MS. Conventional and arealometer fiber measurements were conducted by Starlab Inc., Knoxville, TN, on samples from 25 individual F_5 plants per family. Cottonseed for seed index measurements were collected from hand-picked boll samples from each family in the F_4 generation. One hundred fuzzy seed were counted and weighed to determine an average seed weight for each family.

Seed index is the weight of 100 ginned, but not delinted seed and is an indicator of seed size or density. Lint percent, or lint fraction, is the ratio of lint to the total weight of unginned seed cotton expressed as a percentage. Micronaire is a measure of the fineness of the sample of fibers and is reported in standard micronaire units. Elongation is a measure of the elasticity of the fiber sample. The value is determined at the break point in the strength determination and is defined as a percent stretch of the fiber sample at the breaking point. Strength is the fiber strength of a bundle of fibers measured with two stelometer jaws holding the fiber bundle separated by 0.3175 cm (one-eighth inch).

The digital fibrograph is an instrument for measuring fiber length. Span length is the distance spanned by a specific percentage of the fibers in the test specimen when the initial starting point of the scanning in the test is considered 100%. The 50% span length is the length on the test specimen spanned by 50% of the fibers scanned at the initial starting point. The 2.5% span length is the length on the test specimen spanned by the longest 2.5% of the cotton fibers scanned at the initial starting point. The 2.5% span length approximates the classer's staple.

The arealometer instrument measures the resistance a given mass of fibers offers to the flow of air at two pressures. From these data, other fiber properties such as fineness and shape can be determined and used to calculate immaturity ratio, percentage maturity, perimeter, weight fineness, and wall thickness. The measurement, A, describes the external surface of the fibers of a given volume of fibrous material under standard pressure, expressed in terms of square millimeters per cubic millimeter of fibrous material. The measurement, Ah, measures the same fibers as the A measurement, but under high pressure. The difference between A and Ah is an estimate of the flatness of the fiber ribbon. The greater the difference, the more ribbon-like are the fibers. The immaturity ratio is a dimensionless number that describes a physical characteristic of the fiber cross-section. It is defined as the ratio of the area that the fiber cross-section would have if its perimeter enclosed a circle compared to the area that the perimeter actually encloses.

Measurement of fiber maturity is based on the simple linear regression prediction of the caustic soda percent maturity method (Hertel and Craven,

1951). The prediction equation is M = 150.5 – 38.1I, where I = the calculated immaturity value. The perimeter is defined as the distance around the outside wall of the fiber section in micrometers. The weight fineness, or linear density, is defined as the mass per unit length of fiber expressed in micrograms per inch. The fiber wall thickness is the measurement in micrometers of the width of the wall of the cotton fiber. Equations for calculation of each of these traits and their relationships are given in the National Cotton Variety Test Report by Rayburn et al. (1996).

The total number of nodes is a total of all nodes with the cotyledon node counted as one. Node of first fruiting branch is a physiological trait that gives an indication of earliness and is the node at which the plant develops its first nonvegetative branch. Plant height was measured from ground level to the top of the plant at harvest time. The height/node ratio is obtained by dividing the plant height by the total number of nodes on the plant.

Lint percent measurements were calculated from cotton harvested from individual plants in the F_5 generation. A mean was calculated from individual measurements of 50 plants in each two-row family plot.

Fiber samples from 25 plants per F_2-derived F_5 family were measured twice for each of the fiber traits: micronaire, elongation, strength, 50% span length, 2.5% span length, A, Ah, immaturity, maturity, perimeter, weight-fineness, and wall thickness. A mean was then calculated for each of the traits in each family.

For number of nodes, node of the first fruiting branch, and plant height in the F_5 generation, all plants in the two-row plot were measured individually and a mean was taken from these measurements. White bloom counts were taken in the F_3, once a week, over a 4 week period. A percentage of the plants flowering at a given date for each family was calculated.

Statistical Analysis Methods

To determine if trait data were normally distributed, the skewness and kurtosis values were calculated for each trait. When seeking to detect a QTL between two markers, the other markers linked with some other QTLs are likely to have marker effects, which should be considered in controlling background genetic variation. By employing a mixed model approach where effects of the QTLs are considered fixed and molecular markers are random (Zhu and Weir, 1998) the phenotypic value of a quantitative trait measured on the *j*th individual can be expressed as a mixed linear equation

$$y_j = \mu + ax_{Aj} + dx_{Dj} + \sum_{k \neq i-,i+} e_{Mk} z_{Mkj} + \varepsilon_j \quad [1]$$

where $\boldsymbol{\mu}$ is the population mean; $\boldsymbol{a}$ and $\boldsymbol{d}$ are the additive and dominance effects for the searching QTL; $\boldsymbol{x_{Aj}}$ and $\boldsymbol{x_{Dj}}$ are coefficients for genetic effects; $\boldsymbol{e_{Mk}}$ is the random effect for the $\boldsymbol{k}$th marker genotype with its coefficient $\boldsymbol{z_{Mk}}$ taking the value of 1 for $\mathbf{M_{k1}M_{k1}}$, 0 for $\mathbf{M_{k1}M_{k2}}$, or -1 for $\mathbf{M_{k2}M_{k2}}$; and $\boldsymbol{\varepsilon_j}$ is the random residual effect.

Equation [1] can be rewritten by a matrix form of the mixed linear equation for all the phenotypic values,

$$\mathrm{y} = \mathrm{Xb} + \mathrm{Z}_M \mathrm{e}_M + \mathrm{e}_\varepsilon$$
$$\sim N(\mathrm{Xb},\ \mathrm{v} = \sigma^2_M \mathrm{Z}_M F_M \mathrm{Z'}_M + \sigma^2_\varepsilon \mathrm{I}) \quad [2]$$

where $\mathbf{y}$ is a vector of phenotypic values of quantitative trait studied; $\mathbf{b}$ is a vector of the fixed effects; $\mathbf{X}$ is the coefficient matrix with row vectors $\mathbf{x'}_j$; $\mathbf{e}_M$ is a vector of random effects for markers, $\mathbf{F}_M$ is a constant matrix describing the relationship between markers; $\mathbf{Z}_M$ is the coefficient matrix for $\mathbf{e}_M$, and $\mathbf{Z'}_M$ is the transpose matrix of $\mathbf{Z}_M$; $\mathbf{e}_\varepsilon$ is a vector of random residual effects.

Programs for the mixed equation approach were written in C. The mixed equation approach program calculates the likelihood ratio value for testing the presence of a QTL within linkage groups. The mixed model approach program searches for QTLs along the whole genome by a step of 2.0 cM and also gives estimates of the likelihood ratio value and genetic additive and dominance effects. The distribution of the likelihood ratio is closely approximated by the chi-square distribution, thus, the chi-square distribution values can be used to test for levels of significance in the likelihood ratio. A likelihood ratio value threshold of 6.63 or above was chosen, which provides significance with a probability of 0.01, with one degree of freedom. When the likelihood ratio value equals 7.88 or 10.83, a QTL significantly associated with the

marker is indicated with probability levels of 0.005 or 0.001, respectively. Therefore, if one chooses to use a probability level higher than 0.01 the likelihood ratio values for our data are shown in Table 3. Estimated genetic additive and dominance effects were tested for significance by using the standard normal distribution. Additive and dominance effects are defined with respect to the MAR (multiadversity resistant) allele. Thus, negative genetic effect values indicate that the MAR allele decreases the phenotypic value of the trait, and positive values indicate an increase in the phenotype with MAR alleles. The HS 46 allele has the opposite effect.

RESULTS AND DISCUSSION

Agronomic and Fiber Traits

The phenotypic values of agronomic and fiber traits are presented in Table 1. These traits segregated continuously and both skewness and kurtosis values, except number of nodes (kurtosis value 1.59), suggested that the agronomic and fiber traits in the present study were normally distributed and thus suitable for QTL analysis. Several of the fiber traits were significantly correlated (Table 2). The arealometer and micronaire provide different, but related, measurements of fiber parameters.. The precise ways in which these measurements relate to physical properties of the fiber are not known. The fiber measurements we show are commonly accepted by breeders. Fiber traits that are highly correlated, may represent different measurements of related fiber components. For example, micronaire, A, and Ah are measures of the resistance of a plug of cotton fibers to air flow. As such, the three are necessarily correlated. Micronaire only provides a single measurement of the resistance; whereas, the arealometer provides measures of resistance to air flow at two air pressures. Interpretation of micronaire readings in terms of fineness and/or maturity requires either perimeter or a maturity measure; whereas, fineness and maturity can be calculated from the two arealometer readings.

For this study we chose to measure fiber quality by both instruments as well as measuring fiber strength and elongation by the stelometer. Each trait is useful to the cotton breeder, thus we report data on QTLs for each trait. The group of highly correlated traits, micronaire, A, Ah, immaturity, maturity, wall thickness, and weight fineness are influenced by fiber fineness and maturity. We expected several of the traits or measurements to be influenced by the same QTLs and our data tend to support this. By showing QTLs for each fiber measurement, breeders may be able to obtain a better idea of how the various measures relate to one another at the genetic level. May and Taylor (1998) reported on how these various fiber measurements relate to one another and to selection in a breeding program for improved yarn tenacity.

Table 1. Phenotypic data of agronomic and fiber traits for 96 F_2-derived families from a cross of MARCABUCAG8US-1-88 x HS 46.

Trait	Mean	SD	Maximum	Minimum	Skewness	Kurtosis
Seed index, g	11.1	1.0	13.5	8.2	-0.10	-0.17
Lint fraction, %	35.7	1.3	39.4	33.1	0.33	-0.20
Micronaire	4.24	0.36	4.95	3.29	-0.45	-0.08
Elongation, %	6.72	0.48	7.79	5.57	-0.34	-0.25
Strength, kN m kg^{-1}	203	10	226	176	0.20	-0.12
50% Span length, mm	13.97	0.51	14.99	12.95	-0.04	-0.70
2.5% Span length, mm	28.45	0.76	30.23	26.42	-0.07	-0.40
Ah	498	36	602	424	0.48	0.02
A	471	31	556	409	0.49	-0.02
Immaturity	1.68	0.12	2.04	1.43	0.28	-0.02
Maturity, %	86	5	95	72	-0.30	0.02
Perimeter, µm	44.8	1.7	49.4	40.2	0.02	-0.42
Weight fineness	3.73	0.28	4.34	3.06	-0.20	-0.17
Wall thickness, µm	2.68	0.22	3.25	2.14	0.06	-0.08
Nodes	18.4	1.3	22.3	14.6	0.11	1.59
Node 1st fruiting branch	6.9	0.4	7.9	6.2	0.59	0.25
Height, cm	77.8	6.0	92.1	64.9	-0.09	-0.50
Height/node ratio	4.3	0.4	5.4	3.4	0.14	-0.05
Bloom rate, %	48.9	17.1	92.1	11.7	-0.07	-0.03

Table 2. Correlation coefficients among fiber traits in F_5 generation (seed index in F_4).

	Seed index	Lint fraction	Micron-aire	Elon-gation	Strength 50%	Span length 2.5%	Span length	Ah	A	Imma-turity	Maturity Fineness	Peri-meter	Wt.
Micronaire	**0.50***	**0.13**											
E1ongation	**-0.2**	**0.12**	**-0.41****										
Strength	**-0.26****	**-0.27****	**0.36****	**-0.52****									
50% Span length	**0.29****	**-0.31****	**0.19**	**-0.15**	**0.31****								
2.5% Span length	**0.31****	**-0.38****	**-0.04**	**-0.20***	**0.20***	**0.64****							
Ah	**-0.54****	**-0.08**	**-0.94****	**0.43****	**-0.39****	**-0.14**	**0.02**						
A	**-0.55****	**-0.11**	**-0.95****	**0.40****	**-0.35****	**-0.13**	**-0.05**	**0.99***					
Immaturity	**-0.35****	**0.04**	**-0.76***	***0.53****	**-0.55****	**-0.16**	**-0.1**	**0.89****	**0.85***				
Maturity	**0.36****	**-0.04**	**0.76****	**-0.53****	**0.54****	**0.15**	**0.1**	**-0.89****	**-0.85****	**-0.99***			
Perimeter	**0.23***	**0.25****	**0.15**	**0.34****	**-0.45****	**-0.06**	**-0.25****	**0.03**	**-0.05**	**0.48****	**-0.48****		
Weight fineness	**0.51****	**0.23***	**0.86****	**-0.16**	**0.06**	**0.06**	**-0.21***	**-0.80****	**-0.85****	**-0.45****	**0.45****	**0.57****	
Wall thickness	**0.23***	**-0.09**	**0.91****	**-0.46****	**0.42****	**0.08**	**-0.1**	**-0.98****	**-0.97****	**-0.92****	**0.92****	**0.13**	**0.74**

***,** Significantly different from zero at the 0.05 and 0.01 levels of probability, respectively.**

Table 3. Maximum likelihood locations of agronomic and fiber trait QTLs , likelihood ratio values, and estimates for additive and dominance effects relative to MAR base phenotype. Mixed model analysis of an F_2-derived population of 96 families from a cross of MARCABUCAG8US-1-88 x HS 46.

QTL Trait	Linkage Group	Map Dis† (cM)	LR‡	Add. Effect	± SE	Dom. Effect	±SE
Seed index, g	**4**	**32.5**	**7.64****	**-1.18***	**±0.51**	**-0.44**	**±0.41**
Seed index, g	**11**	**86.5**	**7.79****	**-2.00***	**±0.83**	**-0.91**	**±0.61**
Seed index, g	**14**	**38.5**	**12.03******	**-0.28***	**±0.13**	**0.46**	**±0.25**
Seed index, g	**14**	**54.5**	**9.7*****	**-0.26***	**±0.12**	**0.38***	**±0.18**
Lint fraction , %	**4**	**36.5**	**9.54*****	**0.58****	**±0.19**	**0.79**	**±0.78**
Lint fraction, %	**10**	**66.5**	**8.82*****	**-0.40***	**±0.18**	**0.36**	**±0.34**
Lint fraction, %	**15**	**10.5**	**7.41****	**0.43****	**±0.16**	**0.46**	**±0.34**
Lint fraction, %	**16**	**10.5**	**12.16******	**1.32****	**±0.38**	**1.08***	**±0.42**
Lint fraction, %	**25**	**2.5**	**8.55*****	**1.95***	**±0.87**	**0.63**	**±0.63**
Micronaire	**6**	**6.5**	**7.97*****	**0.95***	**±0.39**	**0.74****	**±0.26**
Micronaire	**7**	**18.5**	**7.03****	**0.99****	**±0.38**	**0.63***	**±0.26**
Micronaire	**9**	**0.5**	**8.25*****	**-0.13****	**±0.05**	**-0.16**	**±0.09**
Micronaire	**10**	**2.5**	**7.76****	**0.30***	**±0.13**	**0.75****	**±0.28**
Micronaire	**11**	**2.5**	**7.86****	**0.37****	**±0.13**	**0.65***	**±0.26**
Micronaire	**14**	**2.5**	**16.00******	**1.07****	**±0.30**	**0.54***	**±0.22**
Micronaire	**14**	**54.5**	**19.75******	**0.11***	**±0.04**	**-0.22****	**±0.07**
Micronaire	**17**	**14.5**	**8.66*****	**0.40****	**±0.14**	**0.81****	**±0.28**
Micronaire	**19**	**50.5**	**7.49****	**0.33***	**±0.14**	**0.69****	**±0.26**
Micronaire	**20**	**8.5**	**7.03****	**0.35****	**±0.13**	**0.65***	**±0.26**
Micronaire	**24**	**0.5**	**7.76****	**1.03****	**±0.37**	**0.70****	**±0.26**
Micronaire	**24**	**50.5**	**10.58*****	**-0.01**	**±0.05**	**0.26****	**±0.09**
Micronaire	**25**	**0.5**	**9.05*****	**1.00***	**±0.38**	**0.51**	**±0.26**
Micronaire	**27**	**0.5**	**7.14****	**0.31***	**±0.13**	**0.68***	**±0.26**
Micronaire	**28**	**6.5**	**6.75****	**-0.02**	**±0.04**	**0.23***	**±0.10**
E1ongation, %	**4**	**30.5**	**9.44*****	**-1.61****	**±0.53**	**-1.08****	**±0.37**
E1ongation, %	**6**	**8.5**	**11.28******	**-1.40****	**±0.47**	**-1.13****	**±0.34**
E1ongation, %	**7**	**18.5**	**13.44******	**-1.58****	**±0.50**	**-0.82***	**±0.34**
E1ongation, %	**10**	**2.5**	**10.75*****	**-0.54****	**±0.17**	**-0.93***	**±0.36**
E1ongation, %	**11**	**0.5**	**12.88******	**-0.59****	**±0.17**	**-1.00****	**±0.34**
E1ongation, %	**14**	**2.5**	**16.93******	**-1.63****	**±0.41**	**-0.93****	**±0.30**
E1ongation, %	**15**	**0.5**	**8.40*****	**-0.45***	**±0.17**	**-1.03****	**±0.36**
E1ongation, %	**16**	**0.5**	**7.63****	**-1.39****	**±0.51**	**-0.96****	**±0.35**
E1ongation, %	**17**	**14.5**	**10.48*****	**-0.50****	**±0.18**	**-1.13****	**±0.36**
E1ongation, %	**18**	**0.5**	**7.98*****	**-0.45****	**±0.16**	**-0.99****	**±0.35**
E1ongation, %	**19**	**48.5**	**11.61******	**-0.48****	**±0.18**	**-1.11****	**±0.35**
E1ongation, %	**20**	**8.5**	**9.57*****	**-0.53****	**±0.17**	**-1.06****	**±0.35**
E1ongation, %	**21**	**0.5**	**9.63*****	**-0.69****	**±0.26**	**-3.43****	**±1.11**
E1ongation, %	**24**	**6.5**	**11.00******	**-1.56****	**±0.52**	**-1.18****	**±0.36**
E1ongation, %	**25**	**0.5**	**10.61*****	**-1.23***	**±0.51**	**-1.04****	**±0.35**
E1ongation, %	**27**	**0.5**	**9.70*****	**-0.45***	**±0.18**	**-1.03****	**±0.34**
E1ongation, %	**28**	**0.5**	**6.84****	**-0.14**	**±0.14**	**-0.41****	**±0.16**
E1ongation, %	**30**	**0.5**	**7.31*****	**-0.44***	**±0.17**	**-0.95****	**±0.35**

Table 3. Continued...

QTL Trait	Linkage Group	Map Dis† (cM)	LR‡	Add. Effect	± SE	Dom. Effect	±SE
Strength, kN m kg^{-1}	6	6.5	6.82**	17	±10.3	16*	±7.1
Strength, kN m kg^{-1}	10	66.5	6.72**	3*	±1.4	-1	±2.6
Strength, kN m kg^{-1}	13	2.5	12.21****	5**	±1.5	0	±3.2
Strength, kN m kg^{-1}	19	56.5	6.97**	6*	±3.2	14*	±5.7
Strength, kN m kg^{-1}	20	2.5	7.15**	8*	±3.2	13*	±6.3
Strength, kN m kg^{-1}	27	4.5	7.96***	5	±3.5	14*	±6.7
50% Span length	6	46.5	8.60***	0.03*	±0.00	0	±0.03
50% Span length	16	16.5	7.45**	-0.03*	±0.00	-0.03*	±0.03
2.5% Span length	3	18.5	9.85***	0.03*	±0.03	0	±0.03
2.5% Span length	12	0.5	7.08**	0.03*	±0.00	0	±0.03
2.5% Span length	16	14.5	7.38**	-0.05*	±0.03	-0.05*	±0.03
2.5% Span length	17	62.5	9.40***	0.03*	±0.00	0.05*	±0.03
2.5% Span length	28	0.5	6.80**	0.05*	±0.03	0.05	±0.03
Ah	9	0.5	7.38**	11.99*	±4.79	17.38	±9.08
Ah	14	2.5	13.84****	-95.88**	±30.16	-45.55*	±21.71
Ah	14	54.5	18.63****	-10.66*	±4.32	21.58**	±6.54
Ah	19	52.5	7.03**	-25.27	±13.51	-58.86*	±24.91
Ah	24	50.5	12.01****	2.47	±4.64	-27.05**	±8.89
Ah	28	4.5	6.66**	2.86	±4.33	-22.13*	±10.24
A	9	0.5	8.09****	10.92**	±4.08	14.62	±7.74
A	10	54.5	6.83**	-20.05	±10.71	-51.72*	±21.60
A	14	4.5	13.34****	-55.73**	±18.75	-20.8	±14.92
A	14	42.5	16.77****	-9.92*	±4.14	17.02*	±8.41
A	19	50.5	6.66**	-23.60*	±11.65	-52.86*	±22.13
A	24	50.5	12.28****	1.89	±3.95	-23.49**	±7.57
Immaturity	14	42.5	12.68****	-0.04*	±0.02	0.05	±0.03
Immaturity	19	54.5	8.65***	-0.06	±0.04	-0.17*	±0.08
Immaturity	24	50.5	7.69**	0.01	±0.02	-0.07*	±0.03
Immaturity	28	4.5	8.03***	0.01	±0.01	-0.08*	±0.03
Maturity, %	14	54.5	13.82****	1.56**	±0.57	-1.98*	±0.87
Maturity, %	19	54.5	8.63***	2.32	±1.68	6.50*	±3.02
Maturity, %	24	50.5	7.86**	-0.48	±0.61	2.64*	±1.16
Maturity, %	28	4.5	8.09***	-0.51	±0.55	2.97*	±1.31
Weight fineness	9	20.5	10.36****	-0.12**	±0.04	-0.09	±0.07
Weight fineness	10	2.5	10.76***	0.18	±0.10	0.55**	±0.21
Weight fineness	14	54.5	13.39****	0.04	±0.03	-0.17**	±0.05
Weight fineness	17	14.5	7.05**	0.28*	±0.11	0.55**	±0.21
Weight fineness	17	16.5	7.97***	0.28**	±0.10	0.57**	±0.20
Weight fineness	24	50.5	10.06***	0	±0.04	0.20**	±0.07
Weight fineness	25	0.5	13.95****	0.73*	±0.29	0.29	±0.20
Wall thickness, µm	6	6.5	7.07**	0.61*	±0.24	0.44**	±0.17
Wall thickness, µm	9	0.5	7.93***	-0.07*	±0.03	-0.12*	±0.06
Wall thickness, µm	10	54.5	7.45**	0.16*	±0.08	0.39*	±0.15
Wall thickness, µm	14	2.5	12.05****	0.57**	±0.18	0.29*	±0.13
Wall thickness, µm	14	54.5	20.64****	0.08**	±0.03	-0.13**	±0.04
Wall thickness, µm	19	52.5	8.20***	0.15	±0.08	0.37*	±0.15
Wall thickness, µm	24	50.5	10.82****	-0.02	±0.03	0.15**	±0.05
Wall thickness, µm	28	6.5	8.03***	-0.03	±0.03	0.12*	±0.06
Nodes	14	54.5	7.14**	0.01	±0.15	-0.63**	±0.24
Nodes	23	0.5	7.55**	-0.22	±0.16	-0.92**	±0.34
Nodes	31	0.5	6.71**	-0.08	±0.16	0.69*	±0.33
Node 1st Fruiting branch	10	22.5	9.06***	-0.01	±0.05	0.27**	±0.09
Height, cm	6	40.5	7.67**	0.61	±0.72	-3.56*	±1.65
Height, cm	23	0.5	10.33***	-2.44**	±0.76	-1.17	±1.64
Height/node ratio	10	8.5	6.71**	-0.07	±0.06	-0.37*	±0.15
Height/node ratio	23	6.5	6.83**	-0.04	±0.05	0.23*	±0.11
B1oom rate, %	7	14.5	7.79**	8.88	±9.41	17.09*	±7.75
B1oom rate, %	7	38.5	9.70***	0.38	±2.22	13.15**	±4.37

, **,, **** Significant at the 0.05, 0.01, 0.005, and 0.001 levels of probability, respectively.**

† Map distance from first molecular marker in linkage group to the estimated location of the QTL.

‡ LR is likelihood ratio of the QTL.

Quantitative Trait Loci and Their Importance

The QTLs identified in this population were tested for acceptance using the likelihood ratio that has approximately the chi-square distribution. Thus, likelihood ratio values of 6.63, 7.88, and 10.82 represent significant values with probabilities of 0.01, 0.005, 0.001, respectively. A high selection threshold such as 0.005 or 0.001 provides strong evidence that the reported QTLs are actually associated with the respective traits. We only report QTLs whose likelihood ratio values were greater than 6.63 and which showed a significant additive or dominance genetic effect. Fiber traits measured in the F_5 generation, on which the majority of the analysis was based, provided an exceptional measurement for the individual family means and variances, because measurements were made on 25 individual plants for each family.

A total of 100 QTLs which mapped to 60 maximum likelihood locations in 24 linkage groups were identified by the mixed model approach (Table 3 and Fig. 1). At least one QTL was identified for each of the 19 agronomic and fiber traits except perimeter. Elongation and micronaire had the largest number of QTLs identified. Highly correlated traits (Table 2) were expected to show similar QTL results in the mixed model analyses (Table 3 and Fig. 1). Traits related to fiber fineness and maturity were significantly correlated and often showed QTLs at the same or very close maximum likelihood locations in a linkage group.

Additive and dominance genetic estimates were also calculated by the mixed model approach (Table 3). The additive and dominance genetic estimates for each trait show the relative importance of the various QTLs for any given trait. For most traits, alleles at different QTLs from either parent could contribute to increased performance for the trait. The genetic estimates of additive and dominance are defined in relation to the MAR parent. Negative genetic estimates indicate that MAR alleles reduce values of traits by the amounts shown for the estimates. Conversely, positive genetic estimates indicate that MAR parent alleles increase values of traits by amounts equivalent to their genetic estimates. The additive or dominance values of the HS 46 alleles on the trait are simply the opposite sign of those shown in Table 3, that is, a negative value indicates that the HS 46 allele will increase the trait by the amount shown.

Data for QTLs for selected individual traits are interesting. For example, a QTL for seed index with an additive genetic effect of 2 g is located in linkage group 11. QTLs for lint percentage with significant additive or dominance effects that could putatively change lint percentage by more than 1%

Table 4. Selected examples of QTLs and genetic effects in linkage groups 14 and 19 which affect traits of interest for breeding upland cotton.

QTL trait	Linkage group	Maximum		Estimate of generic effects			
		Likelihood location	Likelihood ratio	Additive	±SE	Dominance	±SE
Micronaire	14	2.5	16.00****	1.07**	±0.30	0.54*	±0.22
Elongation, %	14	2.5	16.93****	-1.63**	±0.41	-0.93**	±0.30
Ah	14	2.5	13.84****	-95.88**	±30.16	-45.55	±21.71
Wall thickness, µm	14	2.5	12.05****	0.57**	±0.18	0.29*	±0.13
A	14	4.5	13.34****	-55.77**	±18.75	-20.8	±14.92
Seed index, g	14	38.5	12.03***	-0.28*	±0.13	0.46	±0.25
A	14	42.5	16.77****	-9.92*	±4.14	17.02*	±8.41
Immaturity	14	42.5	12.68****	-0.04*	±0.02	0.05	±0.03
Seed index, g	14	54.5	9.74***	-0.26*	±0.12	0.38*	±0.18
Micronaire	14	54.5	19.75****	0.11*	±0.04	-0.22**	±0.07
Ah	14	54.5	18.63****	-10.66*	±4.32	21.58**	±6.54
Maturity, %	14	54.5	13.82****	1.56**	±0.57	-1.98*	±0.87
Weight fineness	14	54.5	13.39****	0.04	±0.03	-0.17**	±0.05
Wall thickness, µm	14	54.5	20.64****	0.08**	±0.03	-0.13**	±0.04
Elongation, %	19	48.5	11.61****	-0.48**	±0.18	-1.11**	±0.35
Micronaire	19	50.5	7.49**	0.33*	±0.14	0.69**	±0.26
A	19	50.5	6.66**	-23.16*	±11.65	-52.86*	±22.13
Ah	19	52.5	7.03**	-25.27	±13.51	-58.86*	±24.91
Wall thickness, µm	19	52.5	8.20***	0.15	±0.08	0.37*	±0.15
Immaturity	19	54.5	8.65***	-0.06	±0.04	-0.17*	±0.08
Maturity, %	19	54.5	8.63***	2.32	±1.68	6.50*	±3.02
Strength, kN m kg -1	19	56.5	6.97**	6	±3.2	14*	±5.7

*, **, ***, **** Significant at the 0.05, 0.01, 0.005, and 0.001 levels, respectively.

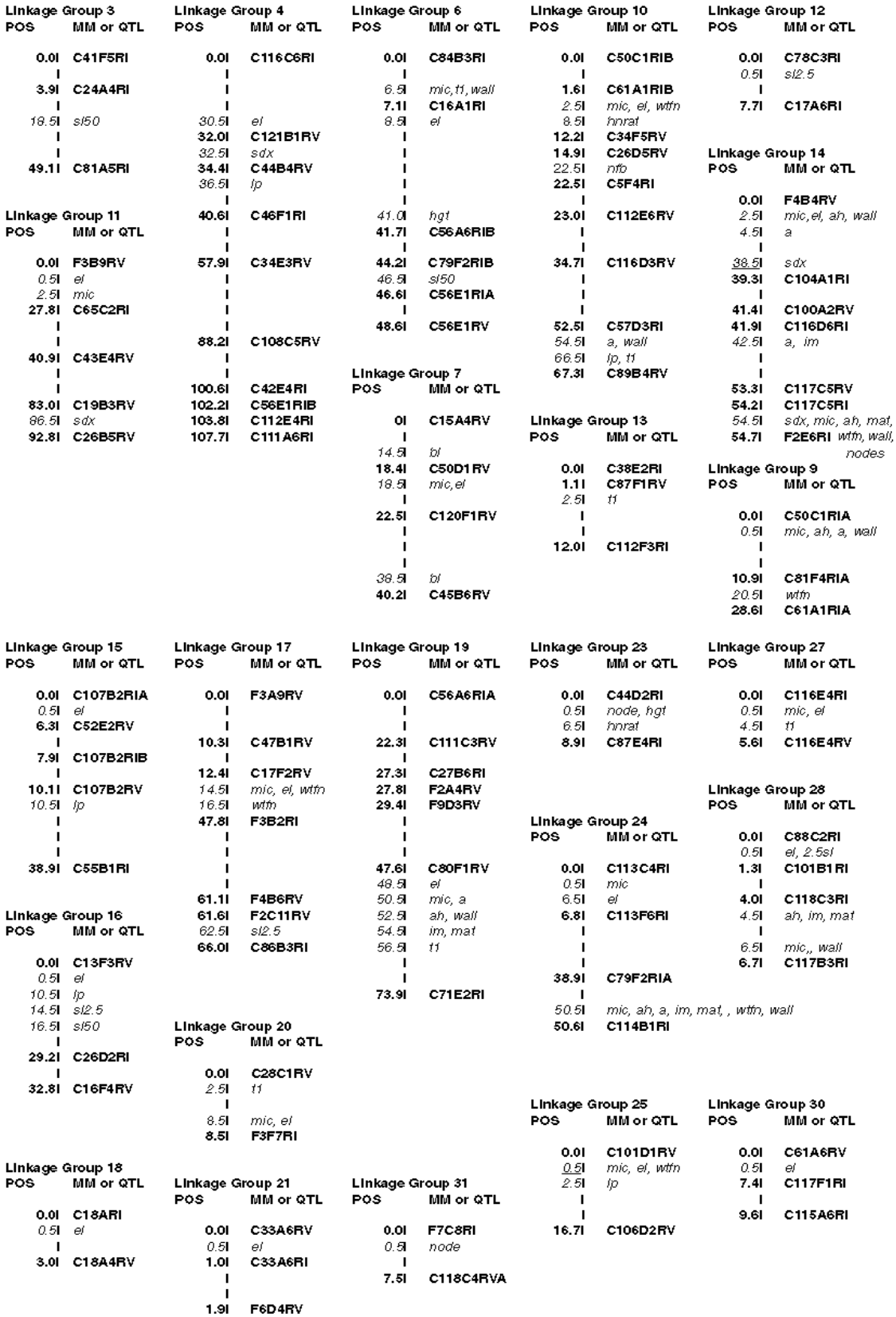

Figure 1. Linkage map of molecular markers and maximum likelihood estimates of location of QTLs. Molecular markers shown in upper case and QTL loci shown in lower case italics.

were detected in linkage groups 16 and 25. Three QTLs in linkage groups 14, 24, and 25 had additive genetic effects of more than 1 micronaire unit. Four QTLs for fiber strength were detected that had dominance effects of more than 9.8 kN m kg^{-1}. Although several QTLs for fiber length were detected, none had a major effect. Two QTLs with major dominance effects on bloom rate were found in linkage group 7. Linkage groups 14 and 19 had several QTLs affecting fiber traits and these two linkage groups were selected to illustrate the influence of QTLs on more than one trait (Table 4).

As more molecular markers are developed and the map is supplemented with finely scaled increments, these QTLs will become more refined in relation to molecular markers. This should make the identification of the QTL more useful to applied breeders as marker-assisted selection should then be more feasible.

The putative locations of the QTLs do not necessarily represent physical distances. Thus, a physical map of the linkage groups is very much needed and would be of great value in cloning-selected QTLs in cotton. This research forms a beginning for progress in understanding QTLs in upland cotton and how they are distributed and/or associated among linkage groups for various traits. It also provides evidence that some QTLs influence several traits and/or several measures of fiber traits in upland cotton as well as showing that some linkage groups, such as 14 and 19, have major QTLs for several useful traits. Research is presently underway in our laboratory to assign these linkage groups to specific chromosomes in the cotton genome by associating specific restriction fragment length polymorphism molecular markers with known cytogenetic chromosome deficiency germplasm lines and, thus, associating these QTLs with specific chromosomes in cotton. This association could target selected chromosomes for further analysis such as the development of chromosome substitution lines with specific chromosomes from other species. Linkage groups 14 and 19 appear to be good candidates for this approach.

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