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Genetic mapping and QTL analysis of fiber-related traits in cotton (*Gossypium*)

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Abstract Cotton, the leading natural fiber crop, is largely produced by two primary cultivated allotetraploid species known as Upland or American cotton (*Gossypium hirsutum* L.) and Pima or Egyptian cotton (*G. barbadense* L.). The allotetraploid species diverged from each other and from their diploid progenitors (A or D genome) through selection and domestication after polyploidization. To analyze cotton AD genomes and dissect agronomic traits, we have developed a genetic map in an F_2 population derived from interspecific hybrids between *G. hirsutum* L. cv. Acala-44 and *G. barbadense* L. cv. Pima S-7. A total of 392 genetic loci, including 333 amplified fragment length polymorphisms (AFLPs), 47 simple sequence repeats (SSRs), and 12 restriction fragment length polymorphisms (RFLPs), were mapped in 42 linkage groups, which span 3,287 cM and cover approximately 70% of the genome. Using chromosomal aneuploid interspecific hybrids and a set of 29 RFLP and SSR framework markers, we assigned 19 linkage groups involving 223 loci to 12 chromosomes. Comparing four pairs of homoeologous chromosomes, we found that with one exception linkage distances in the A-subgenome chromosomes were larger than those in their D-subgenome homoeologues, reflecting higher recombination frequencies and/or larger chromosomes in the A subgenome. Segregation distortion was observed in 30 out of 392 loci mapped in cotton. Moreover, approximately 29% of the RFLPs behaved as dominant loci, which may result from rapid genomic changes. The cotton genetic map was used for quantitative trait loci (QTL) analysis using composite interval mapping and permutation tests. We detected seven QTLs for six fiber-related traits; five of these were distributed among A-subgenome chromosomes, the genome donor of fiber traits. The detection of QTLs in both the A subgenome in this study and the D subgenome in a previous study suggests that fiber-related traits are controlled by the genes in homoeologous genomes, which are subjected to selection and domestication. Some chromosomes contain clusters of QTLs and presumably contribute to the large amount of phenotypic variation that is present for fiber-related traits.

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Introduction

Cotton is the leading natural fiber crop and a major oilseed crop in the world. The cotton genus *Gossypium* includes about 50 species of two polyploidy levels, commonly known as diploid ($2n=2x=26$) or tetraploid ($2n=4x=52$). The diploid species ($2n=26$; genomes A, B, C, D, E, F, G, or K) are distributed tropically and subtropically in a geographically related manner. The A-genome species occur naturally in Africa and Asia, while the D-genome species occur in the New World. Subscripts in each group are used to denote related genomes within a genomic group such as *G. herbaceum* L. (A_1) and *G. aboreum* L. (A_2). The most extensively cultivated cotton species are allotetraploid *G. hirsutum* L. (upland or American cotton) and *G. barbadense* L. ("Egyptian" cotton). Both allotetraploids originated in the New World from interspecific hybridization between species closely related to *G. herbaceum* L. (A_1) or *G. arboreum* L. (A_2) and an American diploid, *G. raimondii* L. (D_5) or *G. gossypioides* (Ulbrich) Standley (D_6) (Beasley 1940). Hybridization and polyploidization are estimated to have

occurred 1–2 million years ago (Wendel et al. 1995), which gave rise to a disomic polyploid consisting of five extant allotetraploid species (Percival et al. 1999). Domesticated tetraploid cottons appeared in the New World by 3,500–2,300 B.C. (Jiang et al. 1998). The cotton fiber production is affected by polyploid formation and crop domestication. The A genome produces spinnable fiber, whereas the D genome alone is worthless in terms of fiber production (Applequist et al. 2001), although it contributes to other agronomic traits. Jiang et al. (1998) found that the majority of quantitative trait loci (QTLs) for fiber-related traits is located in D subgenome and proposed that some genes in the D subgenome in allotetraploids are mutated to produce fibers, which is subsequently subjected to selection and domestication. However, novel gene expression may also result from interactions between homoeologous genomes and the reactivation of suppressed genes in the D subgenome or the enhancement of fiber-producing genes in the A subgenome.

The progress on cotton genome mapping is impeded by relatively large genomes, inadequate DNA markers, and the polyploidy of widely cultivated tetraploid cottons. In spite of these disadvantages, several research groups have developed linkage maps of allotetraploid and diploid cottons and analyzed QTLs for fiber-related traits with respect to cotton domestication (Brubaker et al. 1999; Jiang et al. 1998; Lacape et al. 2003; Reinisch et al. 1994; Shappley et al. 1998; Ulloa and Meredith Jr 2000; Zhang et al. 2002). However, except for an integrated map developed recently (Lacape et al. 2003), many maps are developed independently and provide approximately 10–85% coverage of the approximate 5,500-cM cotton genome (Lacape et al. 2003; Reinisch et al. 1994; Stelly 1993). The DNA markers currently available in cotton are based on restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), and simple sequence repeats (SSRs). There is a need to develop additional markers and integrate linkage maps. Towards this end, we applied amplified fragment length polymorphisms (AFLPs) (Vos et al. 1995) and a set of RFLP and SSR markers to cotton genome analysis. A key feature of the AFLP technology (Vos et al. 1995) is that it permits simultaneous analysis of different DNA regions randomly throughout the entire genome regardless of their origin or complexity. AFLP markers have been extensively used for genetic mapping and QTL analysis in different species (Castiglioni et al. 1999; Klein et al. 2000; Waugh et al. 1997). AFLP in combination with RFLP and SSR analyses is considered to be highly effective in comparative genomic studies (McGregor et al. 2000; Saliba-Colombani et al. 2000).

We developed a cotton genetic map using 333 AFLP, 47 SSR, and 12 RFLP loci in an F_2 population derived from an interspecific cross, *G. hirsutum* L. × *G. barbadense* L. To determine the chromosomal identities and to integrate linkage maps, we employed a set of framework RFLP and SSR markers (Liu et al. 2000; Reinisch et al. 1994; Zhang et al. 2002) and aneuploid interspecific hybrids to assign linkage groups into homoeologous chromosomes. Furthermore, we used linkage maps to analyze QTLs associated with fiber-related traits using QTL CARTOGRAPHER (Basten et al. 2001). Several QTLs for fiber-related traits were readily detected in the homoeologous A subgenome using composite interval mapping and permutation tests (Churchill and Doerge 1994), suggesting that the A subgenome of the allotetraploid cotton contributes to the superior fiber production. The AFLP-markers and linkage maps developed are useful for the comparative analysis of cotton genome evolution and marker-assisted selection of agronomically important traits in breeding programs.

Materials and methods

Plant materials and DNA extraction

We developed an F_2 population of 120 individuals derived from the interspecific hybrids between two cultivated species, namely, *Gossypium hirsutum* L. cv. Acala 44 (AADD, $2n=4x=52$) and *G. barbadense* L. cv. Pima S-7 (AADD, $2n=4x=52$). *G. hirsutum* L. cv. Tamcot Sphinx was used for comparing AFLPs detected within and between species but not used in linkage analysis. Pima S-7 produces fibers 30% longer and 40% stronger than typical *G. hirsutum* cultivars (approx. 1.1 inches of fiber length and approx. 30 grams/tex of fiber strength, respectively), suggesting that there is genetic variation in fiber characteristics between the two cultivars. Phenotypic traits including plant height, presence of leaf and stem hairs, petal color, pollen color, presence of petal spot, number of locules, and boll pitting were determined for each individual plant. When plants matured, seed and cotton samples were harvested from each plant, individually ginned, and scored for seed color, seed weight, number of seeds, lint weight, lint percentage, fiber length, upper quartile length, short fiber content, fiber elongation, fiber strength, fiber fineness, and immature fiber content. The fiber was analyzed at the International Textile Center, Texas Tech University, Lubbock, Texas. Average fiber length was determined by an advanced fiber information system (AFIS). Fiber strength was determined in three replications using the Stelometer instrument and the 1/8th-inch gauge method. A total of 11 fiber-related traits were scored in individual F_2 plants.

The genotypes of polymorphic AFLP and SSR markers were analyzed for 94 F_2 individuals that were fertile or partially fertile and produced fibers. The sterility of plants was probably caused by homozygosity of the asynaptic recessive *as1* and *as2* alleles commonly found in *G. hirsutum* L. and *G. barbadense* L. crosses (Endrizzi et al. 1984). Leaves were collected from labeled plants 42 days after emergence, frozen in liquid nitrogen, and stored at −70°C. DNA was extracted from the parents and 94 F_2 individuals using a modified method (with 2% CTAB and 5% PVP) (Saghai-Maroof et al. 1984). In a large-scale DNA preparation for RFLP analysis, we extracted DNA using a modified protocol as previously described (Paterson et al. 1993).

AFLP fingerprinting

The AFLP procedure was performed as previously described (Vos 1998; Vos et al. 1995) and following the manufacturer's recommendations (AFLP Analysis System I, GIBCO-BRL Life Technologies, Gaithersburg, Md.). Briefly, 300 ng of genomic DNA from each sample was digested with *Eco*RI and *Mse*I followed by ligation of the adapters to the restriction fragments. A primer pair containing the *Eco*RI- and *Mse*I-adapter sequences with one selective nucleotide and random nucleotides was used for pre-

amplification. A total of 144 primer pairs from a combination of nine *Eco*RI and 16 *Mse*I primers (provided by the manufacturer) were used for this study. Selective amplification was performed using primer pairs containing one to three selective nucleotides. One of the *Eco*RI primers was end-labeled by γ-[^{32}P]-ATP or γ-[^{33}P]-ATP using T4 polynucleotide kinase. PCR amplification was carried out in a PTC-225 DNA Engine Tetrad (MJ Research, Waltham, Mass.). The amplified fragments were separated on 6% polyacrylamide sequencing gels that were dried and exposed to X-ray films for detection. Of 144 primer combinations, 55 gave rise to approximately ten or more polymorphic bands each between Pima S-7 and Acala 44 and were selected for F_2 progeny analysis. The polymorphic bands unambiguously segregating in the F_2 population were scored for linkage and QTL analyses. The nomenclature of AFLP loci is indicated by *Eco*RI/*Mse*I primers (3/3 bases) followed by a number with odd numbers for the *G. hirsutum* loci and even numbers for the *G. barbadense* loci. The smallest number represents the largest fragment in the gel.

AFLPs are dominant markers. It is easy to introduce false positives when using the presence or absence of a fragment as a criterion to score a polymorphic band. To minimize the number of false positives, we analyzed the AFLPs in the parental lines as well as their respective interspecific hybrids derived from three allotetraploids (data not shown). If a polymorphic band was present in one of the two parents as well as their F_1 hybrid, we considered the locus detected to be genotype-dependent. Alternatively, a polymorphic band present only in one of the two parental lines but absent in the F_1 hybrids was likely a PCR artifact, so was not included in the F_2 analysis.

RFLP clones, DNA blotting, and hybridization

A set of 192 clones was kindly provided by Andrew H. Paterson, Center for Applied Genomics Technology, University of Georgia (Reinisch et al. 1994). DNA blotting and hybridization were performed as previously described (Chen et al. 1994). Forty-one DNA probes that detected polymorphisms between Acala 44 and Pima S-7 using four enzymes (*Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I) were used for linkage analysis. RFLP analysis indicated that 27 out of the 41 probes (66%) detected polymorphisms between Acala 44 and Pima S-7 using the four restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I). Among them, 15 (37%) detected co-dominant alleles and 12 (29%) dominant alleles.

Simple sequence length polymorphism analysis

SSLPs were amplified in Pima S-7 and Acala 44 in a 15-μl reaction containing 30 ng of template DNA, 0.2 m*M* dNTPs, 3.0 m*M* $MgCl_2$, 0.4 μl of reverse and forward primers (20 μM solution), and 1 U *Taq* polymerase. Forty cycles of PCR were performed using the program of 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min followed by a final extension of 7 min at 72°C. The amplified products were resolved on a 4% Metaphor agarose gel or a 6% polyacrylamide gel. The primers designed to amplify SSRs were either purchased from Research Genetics or kindly provided by Alan Pepper, Biology Department, Texas A&M University (Reddy et al. 2001). Primer sequences are available in http://www.resgen.com/products/ADDMPs.php3 (Liu et al. 2000) and a previous publication (Reddy et al. 2001), respectively. We analyzed approximately 150 SSR primer combinations in the two parental lines. Sixty (40%) of those that amplified polymorphic bands between Pima-S7 and Acala 44 were used to genotype 94 F_2 plants.

Chromosomal assignment of SSR loci

Cytogenetic stocks monosomic for *G. barbadense* L. chromosomes 1–4, 6, 7, 9, 10, 12, 16, 18, 20, and 25 and monotelodisomic for *G. barbadense* L. chromosome arms 1Lo, 2Lo, 3Lo, 4Lo, 5Lo, 6Lo, 9Lo, 10Lo, 10Lo, 12Lo, 14Lo, 15Lo, 18Lo, 20Lo, 22Lo, 25Lo, 2sh, 3sh, 4sh, 6sh, 7sh, 10sh, 16sh, 17sh, 18sh, 20sh, 22sh, and 26sh were used for the chromosomal assignment of SSR loci. These stocks from the Cotton Cytogenetics Collection were evaluated as monosomic or monotelodisomic F_1s between *G. hirsutum* L. cv. Texas Marker 1 (TM1) and *G. barbadense* L. cv. 3-79 (Liu et al. 2000). The chromosomal and genomic nomenclature was based on translocations (Brown 1980; Stelly 1993). Interspecific aneuploid F_1 hybrids between TM1 (aneuploid) and 3-79 (euploid) were used for genotyping SSR markers selected from 17 linkage groups. For a polymorphic locus, the locus was associated with the aneuploid chromosome if the *G. hirsutum* allele (RFLP, SSR, etc.) was absent, whereas the locus was not associated with the chromosome if the *G. hirsutum* allele was present. A linkage group was assigned to a chromosome if several linked markers were associated with a given aneuploid.

Map construction and QTL analysis

Linkage and QTL analyses were performed using MAPMAKER 2.0 (Lander et al. 1987; Lincoln et al. 1992) and QTL CARTOGRAPHER (version 1.15) (Basten et al. 2001), respectively. The QTL analyses were performed using Window version 1.15 and 1.30 and SAS (Moser et al. 1988) Proprietary Software Release 8.1 (SAS Institute, Cary, N.C.). QTL CARTOGRAPHER employs a series of programs to perform different functions for QTL analysis. The critical values for rejection of normality are 5.99 and 9.21 for tests at the 5% and 1% levels, respectively (Basten et al. 2001). Computations using LRMAPQTL were performed to fit the trait data on a linear regression model for each marker. Afterwards, the SRMAPQTL program used stepwise regression to search for QTLs. The forward and backward regression analyses were performed to rank the markers based on their effects on the quantitative trait using the *F*-statistic values. ZMAPQTL, the QTL mapping program, incorporated the information from all of the previously processed programs including QSTAT, LRMAPQTL, and SRMAPQTL. After the composite interval mapping was analyzed by ZMAPQTL, permutation tests (Churchill and Doerge 1994) were performed to estimate experiment-wise specific thresholds. With every permutation performed, phenotypic data were reshuffled against the marker data, and the largest likelihood ratio (LR) statistics over all of the test positions was selected. EQTL was then performed to summarize the results of ZMAPQTL and the permutations. EQTL automatically set the threshold for the LR statistic and selected QTLs corresponding to the LR values determined after running 1,000 permutations.

Results and discussion

AFLP markers and polymorphism levels in cotton

AFLPs were analyzed among three cultivated cotton, namely, *G. barbadense* L. cv. Pima S-7, *G. hirsutum* L. cv. Acala 44, and *G. hirsutum* L. cv. Tamcot Sphinx. The level of AFLPs detected between Pima S-7 and Acala 44 or Tamcot Sphinx was higher than that between Acala 44 and Tamcot Sphinx, indicating that sequence differences between species are greater than those within species. We analyzed approximately 8,800 DNA fragments in the three species using a total of 144 primer combinations. The number of amplified DNA fragments present in a typical PCR reaction ranged from 32 to 111 and averaged approximately 76. For a given primer combination, about 12% of the bands detected were polymorphic between Acala 44 and Pima S-7. The percentage of polymorphism varied from 1.8% to 33% with different primer combi-

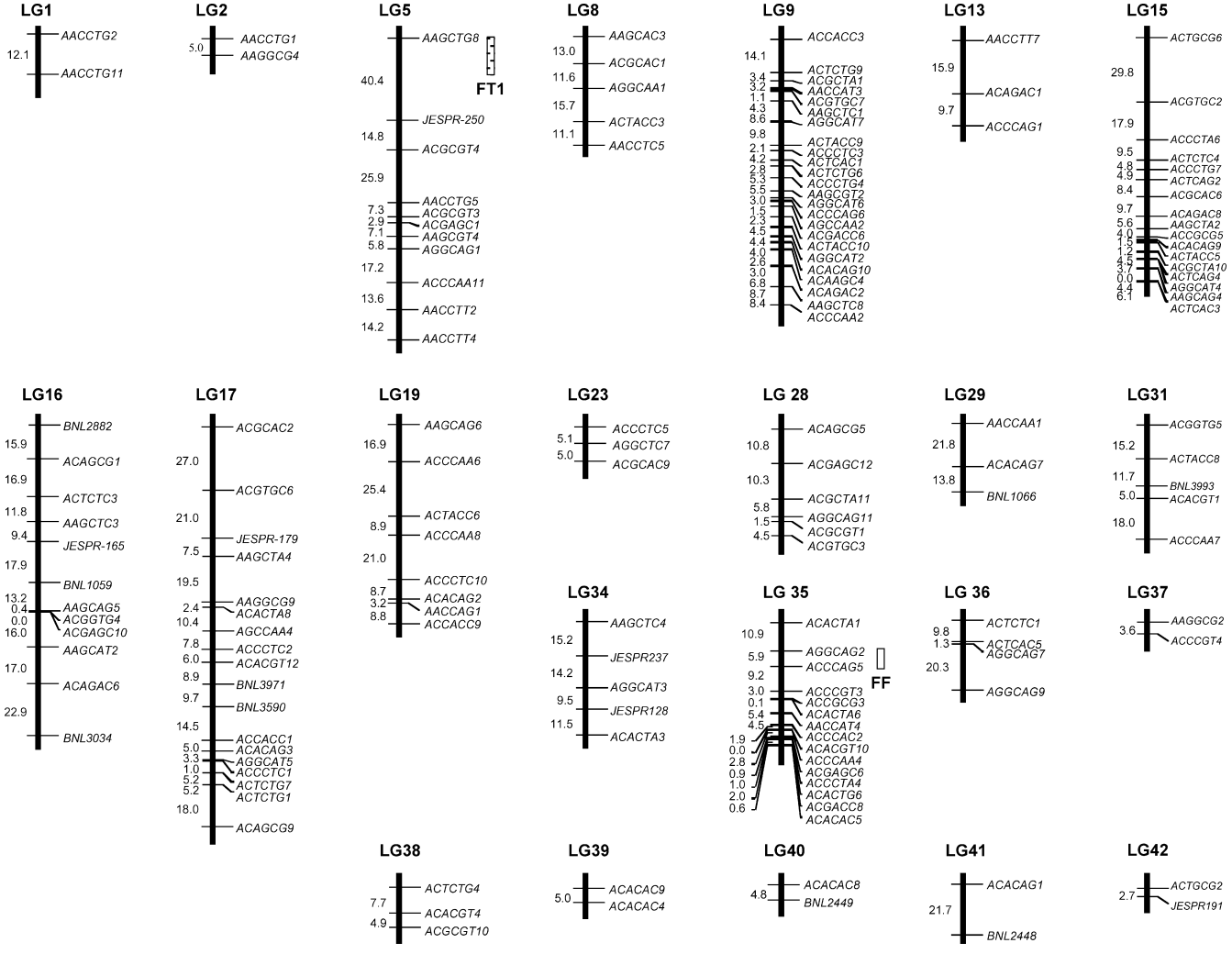

Fig. 1 A genetic map of the cotton genome developed in 94 plants of the F_2 population derived from *Gossypium hirsutum* L. cv. Acala 44 and *G. barbadense* L. cv. Pima S-7. A total of 392 genetic loci, including 333 AFLPs, 47 SSRs and 12 RFLPs, were mapped on 42 linkage groups. The map spans 3,286 cM and covers 70% of the cotton genomes. Map distances (in centiMorgans) between the loci are indicated on the *left side* of the linkage group. The locations of seven QTLs for six fiber-related traits are shown. The loci identified with a chromosomal identity using chromosome aneuploid stocks in this or a previous study (Table 2) served as a set of framework markers that are indicated by *boxes*. The linkage groups are arranged in the *numerical order* of the chromosome numbers (if known) with the homoeologous chromosomes (4 and 22, 6 and 25, 10 and 20, 12 and 26) placed *adjacent* to each other. Linkage groups of unknown chromosome identity are arranged in *numerical order*

nations. The levels of polymorphic fragments detected between Pima S-7 and Acala 44 and Pima S-7 and Tamcot Sphinx were very similar, ranging from 4.0% to 22.8% and 4.0% to 31.9%, respectively.

Over 70% of flowering plants, including many important crops (e.g., wheat, cotton, oat, canola, and peanut) are polyploid (Leitch and Bennett 1997; Masterson 1994; Stebbins 1971). Polyploidy may have evolutionary advantages because duplicated genomes provide additional genetic material for functional divergence and adaptive evolution. Moreover, diploidization of polyploids gives rise to the fixation of homoeologous genes or hybrid vigor. However, diploidization and self-fertilization in many polyploids result in homozygosity for loci within each genome, a process known as an evolutionarily dead-end or genetic "bottleneck" (Stebbins 1940, 1950, 1971). As a result, genetic variation is extremely limited in polyploid species such as *G. hirsutum*, especially among elite cultivated types. Indeed, the level of AFLPs detected within species (approx. 7%) is relatively low compared to that between species (15%).

We selected 53 primer combinations that detected high levels of AFLPs between the two parental lines and their F_1 hybrids for marker segregation tests in 94 F_2 plants derived from interspecific hybrids between Acala 44 and Pima S-7. Approximately 3–15 polymorphic bands could be scored in a given primer combination.

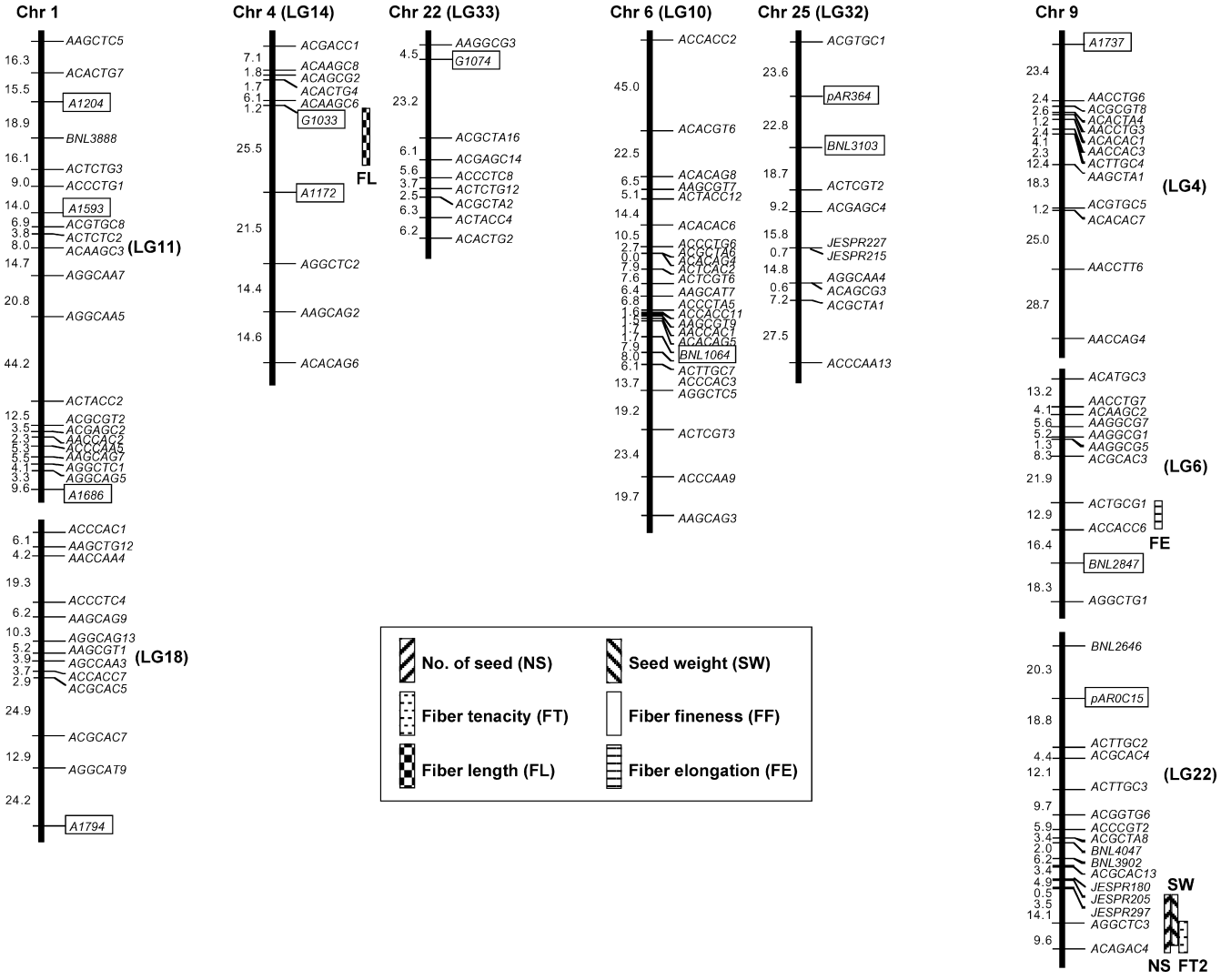

Fig. 1 (continued)

Linkage analysis in cotton

Linkage groups were established using 383 AFLP, 58 SSR, and 27 RFLP loci and MAPMAKER FOR MACINTOSH (version 2.0) (Lander et al. 1987; Lincoln et al. 1992) with a LOD$\geq$3.0. A total of 392 segregating loci, including 333 AFLPs, 12 RFLPs, and 47 SSRs, were assembled into 42 linkage groups (Fig. 1). Sixty-five loci, including 50 AFLPs, 11 SSRs, and 4 RFLPs, displayed polymorphism but could not be assembled into a linkage group (data not shown). An additional 11 RFLPs were not included in the linkage maps, because these loci showed chromosomal locations that were anomalous or inconsistent with the previous study (Reinisch et al. 1994). This is because some RFLP probes detected multiple loci; it was not clear which locus was mapped (Reinisch et al. 1994). Alternatively, segregation distortion and linkage drag may affect locus behavior among different interspecific hybrid populations. The resulting genetic map (Fig. 1) spanned 3,287 cM and covered approximately 70% of the cotton genome based on the estimated map length of approximately 5,500 cM (Lacape et al. 2003; Reinisch et al. 1994; Stelly 1993). The average genetic distance between adjacent mapped loci was 8.4 cM.

Segregation distortion of genetic loci

For the majority of loci mapped in cotton linkage groups, the observed genotypic frequencies did not differ significantly from the expected segregation ratio of 1:2:1 (co-dominant locus) or 3:1 (dominant locus) in the F_2 population. Segregation distortion was significant for 30 loci, including 19 AFLP, 9 SSR and 2 RFLP loci, mapped in 19 linkage groups (Table 1). The majority of loci (approx. two-thirds) with distorted segregation ratios were located in the distal regions of their linkage groups; three were mapped near the central regions and six in the proximal regions. With respect to the origins of allele deficiency, 12 loci showed a deficiency of *G. hirsutum* L.

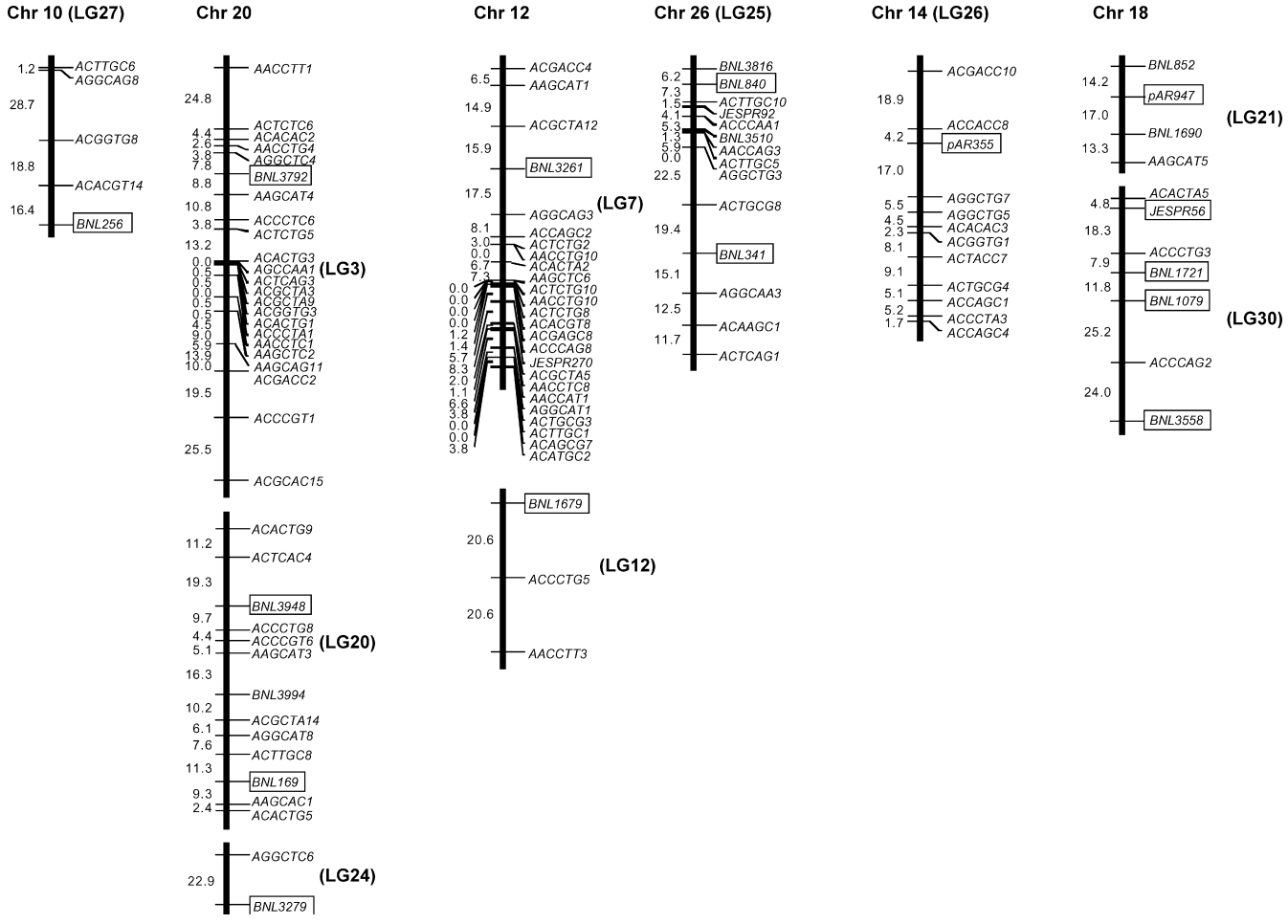

Fig. 1 (continued)

homozygotes, including one region without *G. hirsutum* L. loci, and five regions showed an excess of the *G. hirsutum* L. homozygotes. Eighteen regions showed a deficiency of *G. barbadense* L. homozygotes and seven regions had an excess of *G. barbadense* L. homozygotes.

Assignment of linkage groups to chromosomes

The allele location in the chromosome was determined using genotypes in interspecific aneuploid hybrids between *G. hirsutum* L. (aneuploid) and *G. barbadense* L. (euploid). A linkage group is assigned to the chromosome if several linked markers are associated with a given aneuploid. Fig. 2 shows a panel of aneuploid lines genotyped using a SSR locus (*BNL840*) located in linkage group 25. The aneuploid stocks were maintained as heterozygotes between *G. hirsutum* L. cv. TM-1 and *G. barbadense* L. cv. 3-79. Thus, the SSR marker was heterozygous in all of the lines (except one) and the F_1 hybrids between the two parents, TM-1 and 3-79. In the telosomic line (26sh) in which the long arms of chromosome 26 of *G. hirsutum* L. were absent, only the allele from the parent 3-79 was present, indicating that the *BNL840* allele was located in the long arm of chromosome 26 (Fig. 2). Using this method, we assigned seven

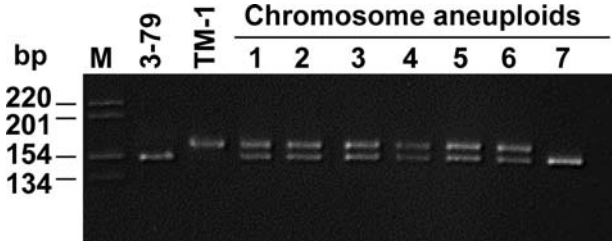

Fig. 2 Chromosomal location is identified for the SSR locus (*BNL840*) using chromosome aneuploid lines. *Lanes 3-79*, *TM-1*, *1* The parental lines and their F_1 hybrid, respectively, *lanes 2–7* alleles amplified from different chromosome aneuploid lines. *Lane 7* contains DNA from monotelosomic 26sh, which lacks *G. hirsutum* or TM-1 alleles in the long arm of chromosome 26. The hemizygous alleles of 3-79 were detected in the 26sh of TM-1 (*lane 7*), suggesting that the *G. hirsutum* SSR allele is located in the long arm of chromosome 26. *Lane M* Molecular size markers

Table 1 Segregation distortion of 30 AFLP, SSR, and RFLP loci in the F_2 population (Acala 44 × Pima S-7)

Markers	Linkage group (chromosome)	Location	Allele deficiency[a]	Segregation[b]				χ^{2c}	*P*
AFLP loci				A or B	C or D		*n*		
Aaggcg4	2	Distal	Gh	31 (A)	58 (C)		89	4.59	0.032
Aaccttl	3 (20)	Distal	Gb	39 (B)	48 (D)		87	18.24	0.000
Aacctg10	7 (12)	Proximal	Gb	14 (A)	75 (C)		89	4.08	0.043
Aagctc6	7 (12)	Distal	Gb	12 (A)	73 (C)		85	5.37	0.021
Acacgt8	7 (12)	Distal	Gb	15 (A)	78 (C)		93	3.90	0.048
Acgagc6	7 (12)	Distal	Gb	14 (A)	76 (C)		90	4.28	0.038
Acgcta5	7 (12)	Distal	Gb	32 (B)	60 (D)		92	4.70	0.030
Aagcag3	10 (6)	Distal	Gh	10 (B)	78 (D)		88	8.73	0.003
Accacc2	10 (6)	Distal	Gb	9 (A)	83 (C)		92	11.36	0.000
Acccaa9	10 (6)	Distal	Gh	6 (B)	80 (D)		86	14.90	0.000
Acagac1	13	Distal	Gh	14 (B)	78 (D)		92	4.70	0.030
Acccag1	13	Distal	Gh	13 (B)	78 (D)		91	5.57	0.018
Aagcag2	14 (4)	Distal	Gb	0 (A)	82 (C)		82	27.33	0.000
Acacag6	14 (4)	Distal	Gb	8 (A)	84 (C)		92	13.04	0.000
Aagcag5	16	Central	Gb	33 (B)	51 (D)		84	9.14	0.002
Accctc4	18 (1)	Proximal	Gh	35 (A)	57 (C)		92	8.34	0.003
Acggtg8	27 (10)	Central	Gb	14 (A)	79 (C)		93	4.91	0.027
Acccag2	30 (18)	Distal	Gb	31 (B)	57 (D)		88	4.91	0.027
Aggcat3	34	Proximal	Gb	33 (B)	44 (D)		77	13.10	0.000
SSR loci				A	B	H	*n*		
BNL2847	6 (9)	Distal	Gb	11	27	37	75	6.84	0.033
BNL1679	12 (12)	Distal	Gh	34	17	34	85	10.20	0.006
BNL3034	16	Distal	Gh	17	12	59	88	10.80	0.005
BNL3590	17	Proximal	Gb	10	20	56	86	10.19	0.006
BNL2646	22 (9)	Distal	Gh	32	8	49	89	13.85	0.000
BNL3816	25 (26)	Distal	Gh	18	13	62	93	10.87	0.004
BNL1066	29	Distal	Gh	28	9	49	86	10.07	0.007
JESRP237	34	Proximal	Gb	11	24	55	90	8.20	0.016
BNL2448	41	Distal	Gh	22	11	52	85	7.09	0.029
RFLP loci				A	B	H	*n*		
A1172	14 (14)	Central	Gb	3	32	55	90	23.13	0.000
pAR947	21 (18)	proximal	Gb	5	13	63	81	26.58	0.000

[a] Indicates deficiency of *G. hirsutum* L. (Gh) or *G. barbadense* L. (Gb) loci
[b] C and A, The polymorphic fragment is present in Pima S-7 (C) but absent in Acala 44 (A); D and B, the polymorphic fragment is present in Acala 44 (D) but absent in Pima S-7 (B); H, heterozygote for Pima S-7 and Acala 44
[c] χ^2,Chi square values were calculated using SAS Proprietary Software Release 8.1 (SAS Institute, Cary, N.C.)

linkages groups into seven chromosomes. An additional 12 linkage groups were assigned to specific chromosomes using a set of 17 SSR and 12 RFLP loci (Liu et al. 2000; Reinisch et al. 1994). Together, 223 loci (180 AFLPs, 32 SSRs, and 12 RFLPs) located in 19 linkage groups were assigned to 12 chromosomes (Fig. 1, Table 2). Linkage groups (LG) 7 and 12 belonged to the same chromosome, as demonstrated by two SSR loci, *BNL3261* in LG7 and *BNL1679* in LG12, which were located in chromosome 12. Similarly, LG3, -20, and -24 were part of chromosome 20; LG4, -6, and -22 belonged to chromosome 9; LG11 and -18 were part of chromosome 1.

Chromosomal rearrangement and polyploidy may affect the statistical interpretation of segregation data and linkage inferences (Doerge and Craig 2000; Livingstone et al. 2000). First, linkage analysis is complicated by extensive recombination per homologous chromosome and the genetic redundancy associated with polyploidy (Brubaker et al. 1999; Reinisch et al. 1994). Some chromosomes may contain recombination hotspots, resulting in large genetic distances, whereas others contain a large portion of heterochromatin associated with low recombination frequencies. Second, interspecific hybrids result in a wide range of phenotypic segregation and fertility. F_1 hybrids are semi-sterile, and one-third of the plants in the F_2 and subsequent generations do not produce any bolls or seeds. Gametic and seed lethality is probably the cause of segregation distortion and unmapped loci, which makes it difficult to develop a high-resolution genetic map using interspecific hybrid-derived populations. Third, genetic redundancy makes it difficult to develop polymorphism-based DNA markers, such as RFLPs, AFLPs, and single nucleotide polymorphism (SNPs). We showed that 65 out of 457 loci (14%), including 50 AFLPs, 11 SSRs, and four RFLPs, displayed polymorphism but could not be assembled into a linkage group. Moreover, 12 out of 41 RFLPs (approx. 29%) are dominant markers. A high number (approx. 36%) of dominant RFLP loci were also detected by Reinisch et al. (1994): 17.3% of the RFLP loci showed the presence or absence of *G. hirsutum* loci and 18.6% for the *G. barbadence* loci. Ulloa and Meredith (2000) detected 97

Table 2 Assignment of linkage groups to chromosomes using anchored RFLP markers, informative SSR loci, and cytogenetic analysis

SSR or RFLP loci[a]	Linkage group and distance (centiMorgans)	Number of markers in linkage group	Chromosomal location	Restriction site (for RFLP loci)	Reference
BNL3792	3 (170.3)	38	20Lo (D)	–	Liu et al. 2000
A1737	4 (124.0)	13	9 (A)	*Eco*RI	Reinisch et al. 1994
BNL2847	6 (107.2)	11	9 (A)	–	This study
BNL3261	7 (113.8)	25	12sh (A)	–	This study
BNL1064	10 (239.9)	24	6sh (A)	–	Liu et al. 2000
A1204	11 (234.3)	29	1 (A)	*Eco*RI	Reinisch et al. 1994
A1593	11 (234.3)	29	1 (A)	*Hin*d III	Reinisch et al. 1994
A1686	11 (234.3)	29	1 (A)	*Eco*RI	Reinisch et al. 1994
BNL1679	12 (41.2)	3	12 (A)	–	Liu et al. 2000
A1172	14 (93.9)	10	4 (A)	*Eco*RV	Reinisch et al. 1994
G1033	14 (93.9)	10	4 (A)	*Eco*RI	Reinisch et al. 1994
A1794	18 (123.8)	13	1 (A)	*Eco*RV	Reinisch et al. 1994
BNL3948	20 (112.9)	13	20 (D)	–	This study
BNL169	20 (112.9)	13	20sh (D)	–	Liu et al. 2000
pAR974	21 (44.5)	4	18 (D)	*Eco*RV	Reinisch et al. 1994
pAROC15	22 (118.8)	16	9 (A)	*Hin*dIII	Reinisch et al. 1994
BNL3279	24 (22.9)	2	20Lo (D)	–	This study
BNL341	25 (112.8)	14	26Lo (D)	–	This study
BNL840	25 (112.8)	14	26Lo (D)	–	This study
pAR335	26 (81.6)	12	14 (D)	*Hin*dIII	Reinisch et al. 1994
BNL256	27 (65.1)	9	10Lo (A)	–	Liu et al. 2000
BNL1079	30 (92.0)	7	18 (D)	–	This study
BNL1721	30 (92.0)	7	18 (D)	–	This study
BNL3558	30 (92.0)	7	18 (D)	–	Liu et al. 2000
BNL3558	30 (92.0)	7	18Lo (D)	–	This study
JESPR56	30 (92.0)	7	18 (D)	–	This study
BNL3103	32 (140.9)	11	25sh (D)	–	Liu et al. 2000
pAR364	32 (140.9)	11	25 (D)	*Xba*I	Reinisch et al. 1994
G1074	33 (58.1)	9	22 (D)	*Xba*I	Reinisch et al. 1994

[a] The suffix of "BNL" or "JESPR" indicates SSR loci; the other suffixes indicate RFLP loci. All of the loci are indicated by boxes in the linkage maps shown in Fig. 1. Lo, long arm; sh, short arm

RFLPs loci using 90 cDNA probes, of which 36 segregated as co-dominant and 61 as dominant loci. The high number of dominant loci may result from rapid genomic changes (deletion, insertion, and other chromosome rearrangements), as reported in *Brassica* and wheat polyploids (Feldman et al. 1997; Ozkan et al. 2001; Song et al. 1995), although such changes were not detected using extensive AFLP analysis in cotton diploids and resynthesized polyploids (Liu et al. 2001). Alternatively, the homoeologous loci are not detected because of co-migrating duplicate DNA fragments (Reinisch et al. 1994) or simply due to PCR artifacts (in the case of AFLP and SSR loci).

The presence of distorted segregation, recombination hotspots, and suppression regions suggest that the cotton genome is complex, making it even more of a challenge to identify and select a gene using a map-based approach. A possible solution is to develop a consensus map using a set of framework markers. Several cotton genetic maps have been developed using RFLPs, SSRs, and RAPDs in the analysis of $F_{2,3}$ populations of *G. hirsutum* L. (Ulloa et al. 2002) and a doubled haploid population (Zhang et al. 2002) or F_2 populations (Reinisch et al. 1994; Ulloa and Meredith Jr 2000) derived from interspecific hybrids between *G. hirsutum* L. and *G. barbadense* L. There is a need to integrate genetic maps independently developed using different mapping populations. Ulloa et al. (2002) successfully integrated genetic maps developed from four mapping populations of upland cotton (*G. hirsutum* L.) using a set of framework RFLP loci. We employed a set of 12 RFLP and 17 SSR loci (Liu et al. 2000; Reinisch et al. 1994) for linkage analysis. The value of framework markers will be enhanced if locus information (fragment sizes, restriction enzymes, primers, etc.) is fully disclosed in each map. Indeed, framework markers have provided an effective means for integrating several genetic maps in cotton (Lacape et al. 2003) and oats (Wight et al. 2003).

Linkage comparison of homoeologous chromosomes

Allotetraploid cotton has 26 pairs of chromosomes: 13 large chromosomes from the A subgenome and 13 small chromosomes from the D subgenome. In a comparison of the genetic maps between homoeologous chromosomes (4 and 22; 6 and 25; 10 and 20; 12 and 26) (Fig. 1), we found that the genetic distances between mapped loci in the A-subgenome chromosomes were often larger than those in homoeologous chromosomes in the D subgenome, which is probably caused by a large amount of repetitive DNA and heterochromatin in the A-subgenome chromosomes (Brubaker et al. 1999; Geever et al. 1989; Kimber 1961; Zhao et al. 1998). The expansion of linkage groups was observed in chromosomes 1, 4, 6, 9, 10, and 12

(A subgenome) (Fig. 1). Chromosomes 1 and 9 each contain three linkage groups. If verified, the total genetic distance is over 300 cM, excluding the gaps of unknown length between the linkage groups. An exception is the homoeologous pair of chromosomes 10 (A subgenome) and 20 (D subgenome). The latter had a larger distance (approx. 300 cM) than the former, which is possibly a consequence of finding fewer markers for chromosome 10. These data are different from those of Reinisch et al. (1994), in which similar genetic distances of RFLP loci were found between A- and D-subgenome chromosomes. A possible explanation is that AFLP loci are often associated with intergenic sequences compared to RFLPs that are derived from coding sequences (e.g., cDNAs) (Saliba-Colombani et al. 2000; Sebastian et al. 2000). Thus, our genetic map using AFLP markers may provide some unique information relevant to chromatin in intergenic sequences that may cause differences in recombination frequencies compared to genic sequences.

Table 3 Mean and standard deviation (SD) for the fiber-related traits examined

Trait[a]	Abbreviation	Mean	SD
Lint weight (per plant)	LW	2.72	0.30
Lint percent (per cotton boll)	LP	28.33	6.62
Seed weight (per plant)	SW	6.42	1.98
Number of seeds (per plant)	NS	50.36	10.17
Upper quartile length (by weight)	UQ	1.26	0.09
Short fiber content (by weight)	SF	6.93	1.74
Fiber length	FL	1.04	0.06
Fiber elongation	FE	7.10	0.38
Fiber tenacity	FT	25.59	2.91
Fiber fineness	FF	136.06	12.33
Immature fiber content	IF	8.11	1.63

[a] Fiber length (FL), The average of the longest 50% of the fibers in the sample; fiber elongation (FE), the amount that a fiber sample stretches prior to breakage and expressed as a percentage; fiber tenacity (FT), fiber strength measured as grams per tex; fiber fineness (FF), measure of the maturity and/or the fineness of fiber and reported in micronaire units; immature fiber content (IF), percentage of fibers with less than 0.25 circularity; the lower the IF, the better the fiber is for dying

Statistical analysis of fiber-related traits

To test the utility of the cotton genetic maps, we analyzed 11 fiber-related traits in the F_2 population using the fiber data collected (Table 3). A large amount of variation for the traits studied was detected. Seed weight was the most variable trait, showing more than eight-fold differences among the 94 plants of the F_2 population studied. Four- to five-fold differences were detected for lint weight, lint percentage, and number of seeds. The least variable traits were fiber length and fiber elongation, which showed only a 25–30% (approximately) difference between the lowest and highest values in the F_2 population. All traits except for number of seeds and lint weight showed normal distribution. The mean values of the traits that did not show normal distribution were converted using square root transformations for QTL analysis, as previously described (Jiang et al. 1998).

Correlation analysis indicated that a number of traits were positively associated with each other (Table 4). Lint weight was significantly correlated with lint percentage, seed weight, and number of seeds, as was the lint percentage with seed weight, number of seeds, and fiber tenacity. The highest correlation was between lint weight and number of seeds (r=0.959), followed by seed weight and number of seeds and lint weight and seed weight. However, an insignificant correlation was found between fiber length and fiber elongation, fiber fineness and fiber length, and fiber elongation and fiber tenacity. A negative correlation was detected between fiber tenacity and seed weight, number of seeds, or fiber elongation (Table 4). Upper quarter length (top 25% of fiber length) had a significantly positive correlation with fiber length (0.331); however, short fiber by weight had a negative correlation with fiber tenacity and fiber fineness.

Analysis of QTLs for fiber-related traits

We employed composite interval mapping (Basten et al. 2001) to detect QTLs for fiber-related traits. A total of 28 QTLs for 11 fiber-related traits were identified using default values of LR ($\geq$11.50) or LOD ($\geq$3.0) (data not shown). However, only seven QTLs remained significant after 1,000 permutation tests were performed for each

Table 4 Statistical tests of correlation coefficients among the fiber-related traits

Traits[a]	LW	LP	SW	NS	FL	FE	FT	FF	UL	SF
LP	0.48**									
SW	0.95**	0.24*								
NS	0.97**	0.36**	0.97**							
FL	−0.07	0.00	−0.02	−0.01						
FE	−0.14	−0.04	−0.10	−0.10	0.07					
FT	−0.17	0.25*	−0.24	−0.23	0.12	−0.31*				
FF	0.04	−0.02	−0.02	−0.01	−0.12	−0.07	0.02			
UL	−0.20	0.03	−0.17	−0.25	0.33*	−0.18	0.29*	−0.12		
SF	−0.04	−0.20	0.03	0.10	0.05	0.11	−0.25*	−0.24	−0.12	
IF	−0.18	−0.15	−0.07	0.00	0.37*	−0.02	−0.20	−0.40**	0.11	0.63**

*, ** indicate that the correlation is significant at the 0.05 and 0.01 probability levels, respectively
[a] See Table 3 for abbreviations

Table 5 Composite interval mapping of QTLs influencing fiber-related traits in cotton (*Gossypium*)(*nd* not determined)

Trait	QTL	Linkage	Chromosome	Subgenome	Marker Interval	LR (LOD)	D[a]	A[b]	PVE[c] (%)
Number of seeds	NS	22	9	A	*JESPR297-acagac4*	19.49 (4.42)	1.32	1.66	22.00
Fiber length	FL	14	4	A	*G1033-A1172*	16.17 (3.50)	0.06	0.01	24.00
Seed weight	SW	22	9	A	*JESPR297-acagac4*	23.85 (5.17)	0.60	0.51	23.00
Fiber tenacity	FT1	5	nd	n.d.[1]	*aagctg8-JESPR250*	17.49 (3.79)	–0.66	2.91	40.00
	FT2	22	9	A	*aggctc3-acagac4*	17.49 (3.79)	0.87	–1.86	31.00
Fiber Fineness	FF	35	nd	n.d.[1]	*aggcag2-acccag5*	23.55 (5.11)	–0.83	–0.19	43.20
Fiber elongation	FE	6	9	A	*actgcg1-accacc6*	23.83 (5.16)	0.56	0.14	42.00

[a] D, Dominant effect
[b] A, Additive effect
[c] PVE, Phenotypic variance explained

trait to determine the significant threshold for the LR statistics (Fig. 1, Table 5). Three QTLs associated with three different traits [number of seeds (NS), seed weight (SW), fiber strength (FT2)] were clustered in the same region of chromosome 9. NS and SW may represent one QTL that has pleiotropic effects on the number of seeds and seed weight. Five of the seven significant QTLs detected are located in the chromosomes of the A subgenome that contributes to fiber production, whereas D-genome species barely have any spinnable fibers (Applequist et al. 2001). The locations for other two QTLs were not determined.

There is a little overlap with respect to the QTLs detected in our research and those of a previous study (Jiang et al. 1998). In the latter, 14 QTLs detected using MAPMAKER QTL (LOD=3.0) were located in the D subgenome and four in the A subgenome. The QTLs detected in both the A and D subgenomes suggest that fiber-related traits result from gene expression and interaction between homoeologous A and D subgenomes. Genome duplication and polyploidization may contribute to novel variation (Osborn et al. 2003; Wendel 2000) associated with fiber development. The combination of A and D genomes stimulates the production of fibers superior to those produced by the A-genome progenitor species (Applequist et al. 2001). It is conceivable that some genes associated with fiber development are suppressed in the D-genome diploid but de-repressed after the combining of the A and D genomes in allotetraploids. Alternatively, the expression of genes in the A subgenome is enhanced because of interactions between homoeologous chromosomes. Furthermore, using permutation tests to determine the critical likelihood ratio value or LOD score for each trait, we improved the efficiency and liability of the QTLs detected in this study (see below). The critical likelihood ratio ranged from 17.49 to 23.85 and the LOD value from 3.50 to 5.17 among seven QTLs associated with six fiber-related traits. The high levels of phenotypic variance (22.0–43.2%) explained by the QTLs indicate that these QTLs have large genetic effects.

Liability of QTL analysis

QTL detection is a statistical test of an association of a trait with a genetic locus or maker interval using maximum likelihood (Lander and Botstein 1989), regression (Haley and Knott 1992), marker regression (Kearsey and Hyne 1994), simple interval mapping (SIM) (Tinker and Mather 1995), and composite interval mapping (CIM) (Zeng 1994). Although every method has some limitations and biases, the results obtained from different methods are often similar when the heritability of a QTL is high (Hyne et al. 1995). When the traits are complex and have relatively low heritability, interval mapping is intuitively attractive. While an ideal model should contain the effects from all of the QTLs, the locations of all the QTLs are often unknown. The CIM procedure (Jansen and Stam 1994; Zeng 1994) employs SIM of a QTL and analysis of variance for other QTLs using partial regression coefficients. CIM is more powerful than other QTL mapping procedures because it gives more power and precision than SIM as the effects of other QTLs are not treated as residual variance. Furthermore, CIM can reduce the bias that would normally be associated with a QTL that is linked to the position being tested.

A common practice for QTL detection employs LOD score threshold (LOD$\geq$2 or 3) (Lander and Botstein 1989) to ensure a 5% overall false positive error for detecting QTLs, which may be a factor for detecting unreliable QTLs (Hyne et al. 1995; Kearsey and Farquhar 1998). If a default LR ($\geq$11.50) or LOD score ($\geq$2.50) was set for detecting a QTL, a total of 28 QTLs for 11 fiber-related traits were identified (data not shown). However, using CIM and permutation tests, we detected only seven QTLs associated with six traits in an F_2 population (*G. hirsutum* L. × *G. barbadense* L.). The main feature of the permutation tests is to generate empirical threshold LR values for each trait in a genome-wide search for QTLs (Churchill and Doerge 1994). The critical threshold values are specific to the experiment and valid for QTL detection. It is evident that each QTL detected has large genetic effects. Notably, although the number of QTLs detected is limited, the majority of QTLs are located in the A-subgenome chromosomes, suggesting a significant role of the A subgenome in fiber development and production during cotton domestication.

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