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M. Ulloa · W.R. Meredith Jr · Z.W. Shappley
A.L. Kahler

RFLP genetic linkage maps from four $F_{2.3}$ populations and a joinmap of *Gossypium hirsutum* L.

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Abstract An RFLP genetic linkage joinmap was constructed from four different mapping populations of cotton (*Gossypium hirsutum* L.). Genetic maps from two of the four populations have been previously reported. The third genetic map was constructed from 199 bulk-sampled plots of an $F_{2.3}$ (HQ95–6×'MD51ne') population. The map comprises 83 loci mapped to 24 linkage groups with an average distance between markers of 10.0 centiMorgan (cM), covering 830.1 cM or approximately 18% of the genome. The fourth genetic map was developed from 155 bulk-sampled plots of an $F_{2.3}$ (119–5 sub-okra×'MD51ne') population. This map comprises 56 loci mapped to 16 linkage groups with an average distance between markers of 9.3 cM, covering 520.4 cM or approximately 11% of the cotton genome. A core of 104 cDNA probes was shared between populations, yielding 111 RFLP loci. The constructed genetic linkage joinmap from the above four populations comprises 284 loci mapped to 47 linkage groups with the average distance between markers of 5.3 cM, covering 1,502.6 cM or approximately 31% of the total recombinational length of the cotton genome. The linkage groups contained from 2 to 54 loci each and ranged in distance from 1.0 to 142.6 cM. The joinmap provided further knowledge of competitive chromosome arrangement, parental relationships, gene order, and increased the potential to map genes for the improvement of the cotton crop. This is the first genetic linkage joinmap assembled in *G. hirsutum* with a core of RFLP markers assayed on different genetic backgrounds of cotton populations (Acala, Delta, and Texas plain). Research is ongoing for the identification of quantitative trait loci for agronomic, physiological and fiber quality traits on these maps, and the identification of RFLP loci lineage for *G. hirsutum* from its diploid progenitors (the A and D genomes).

Keywords *Gossypium hirsutum* L. · RFLP · Restriction fragment length polymorphism · Genetic map · Linkage · Upland cotton · Delta cotton · Texas high plain cotton

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M. Ulloa (✉) · W.R. Meredith Jr
USDA-ARS, Crop Genetics and Production Research Unit, Stoneville, MS 38776, USA
e-mail: mulloa@ars.usda.gov
Tel.: +1-662/686-5464, Fax: +1-662/686-5218

Z.W. Shappley
Monsanto, 700 Chesterfield Parkway N. GG6A, St. Louis, MO 63198, USA

A.L. Kahler
Biogenetic Services Incorporated, 801 32nd Avenue, Brookings, SD 57006, USA

Introduction

Molecular linkage maps based upon DNA markers are widely recognized as essential tools for genetic research in many species. Restriction fragment length polymorphism (RFLP) maps have been employed to document competitive chromosome arrangement in several closely related crops, to further the knowledge of parental relationships (Brubaker et al. 1999), to locate genes for insect and disease resistance (Giese et al. 1993), and for the identification of quantitative trait loci (QTLs) (Paterson et al. 1991; Lee et al. 1996; Paterson et al. 1998; Ulloa and Meredith 2000). The genetic maps developed through interspecific hybridization of cotton currently have little use in a conventional breeding program (Reinisch et al. 1994; Yu et al. 1998). While crosses among races or species show more polymorphism, this polymorphism may not be uniformly distributed across the chromosomes due to the introgression that has ocurred as a result of natural processes or designed breeding programs (Stephens et al. 1967). The inherent problems in such maps are segregation distortions and non-representative genetic distances and linkage relationships (Grant 1975). Distortions in linkage maps are also possible due to minor chromosomal rearrangements that have little effect on fertility or chromosome pairing in meiosis (Slocum et al. 1990). This has also been observed in *Gossypium hirsutum*×*Gossypium barbadense*

L. progeny (personal communication by Dr. Percy). In *G. hirsutum*, two RFLP genetic linkage maps have been developed and used to identify QTLs for agronomic and fiber quality traits (Shappley et al. 1998; Ulloa and Meredith 2000).

Cotton (*Gossypium* spp.) is the world's most-utilized natural textile fiber. This genus comprises about 50 diploid and tetraploid species (Fryxell 1984). Two tetraploid species, *G. hirsutum* and *G. barbadense*, account for 90% and 5% of the world cotton production, respectively (Wendel et al. 1992). The leading modern fiber crop was formed through polyploidization events (Reinisch et al. 1994; Jiang et al. 1998). The modern cotton *G. hirsutum* is comprised of the A and D genomes, (c = 1.8 to 2.5 pg; Arumunganathan and Earle 1991). The D genome, *Gossypium raimondii* Ulbrich, is smaller (c = 0.68 pg) than the A genome, *Gossypium herbaceum* L., (c = 1.05 pg) (Geever et al. 1989).

A major effort is underway to map the cotton genome and to map genes for yield components and fiber quality traits (Reinisch el at. 1994; Shappley et al. 1998; Ulloa and Cantrell 1998; Yu et al. 1998; Jiang et al. 2000; Ulloa and Meredith 2000; Ulloa et al. 2000). There is a need to join the various genetic maps generated around the world (Brubaker et al. 2000).

This study was conducted to provide a framework for mapping of the tetraploid cotton (*G. hirsutum*) genome by presenting the first joinmap from a series of different intraspecific populations with different genetic backgrounds (Acala, Delta, and Texas plain cottons). A core of RFLP marker pairs was used to assemble this initial RFLP joinmap. This study presents the construction of two additional RFLP maps from intraspecific hybridizations, and the first genetic linkage joinmap. The genetic maps will be used to identify QTLs for agronomic, physiological and fiber quality traits, as well as the identification of RFLP loci lineage for *G. hirsutum* from its diploid progenitors (the A and D genomes).

Materials and methods

Mapping populations

In our initial mapping population (Pop 1) previously reported, the breeding line MARCABUCAG8US-1–88 (MAR) and the cultivar 'HS46' were chosen as parental lines (Shappley et al. 1998). Pedigree as well as agronomic fiber quality data showed great diversity between the two parents (Calhoun et al. 1997; Shappley et al. 1998). Ninety six bulk-sampled plots from an $F_{2.3}$ (HS46×MAR) population were used to isolate DNA for genetic mapping. The raw RFLP molecular data (scored bands) was used to develop the cotton joinmap.

Two distinct cotton cultivars from different USA geographical growing regions were used to develop the second mapping population (Pop 2) (Ulloa and Meredith 2000). The 'MD5678ne' parent was a BC_4F_4 plant selection from the recurrent parent 'DES 56', a Delta cultivar with high yield, and low fiber strength and fineness. 'DES 56' was used as a parent for many of the currently used Delta varieties, e.g., 'DES 119', 'DPL 50', 'STV 474', etc. (Calhoun et al. 1997). The 'Prema' parent was a Western cultivar with low yield, and high fiber strength and fineness when grown at Stoneville in the Mississippi Delta. One hundred and nineteen bulk-sampled plots from an $F_{2.3}$ (MD5678ne×Prema) population were used to isolate DNA for genetic mapping. The complete set of RFLP molecular data (scored bands) from each population was used to develop the cotton joinmap.

Two distinct cotton cultivars (HQ95–6 and 'MD51ne') from different USA geographical growing regions were used to develop the third mapping population (Pop 3). Parent HQ95–6 was a single-plant selection from 'Tamcot HQ95', developed from a cross between 'Tamcot CD3H' and MAR-CABU'CS-2–1-83. Tamcot HQ95 had a cylindrical-shaped growth habit and storm-resistant bolls typical of the Texas plain cotton (EL-Zik and Thaxton 1990). The 'MD51ne' parent generally had a high yield and high fiber strength, and low fineness. MD51ne was a BC_2F_2 plant selection that originated from a cross of MD65–11 and 'Deltapine 90' (Meredith 1993). One hundred and ninety nine bulk-sampled plots from an $F_{2.3}$ (HQ95–6×MD51ne) population were used to isolate DNA for genetic mapping.

The fourth population (Pop 4) was developed using two different cultivar types. 'DES 119–5' was a BC_4F_4 plant selection derived from a backcross program to insert the sub-okra leaf trait (*L*) and nectariless 2(ne_1,ne_2) into a Delta cultivar, Des 119. Cultivar DES 119 originated from a cross between 'DES 24' (Reg. no. 69 and P.V. no. 780040) and DES 2134–047. DES 2134–047 was a sister line of 'DES 56' (Reg.no. 70 and P.V. no. 780041). DES 119 is an early maturing, rapid-fruiting cotton that possesses a semi-cluster or short-fruiting-branch plant structure (Bridge 1986). The other parent was MD51ne (Meredith 1993). One hundred and fifty bulk-sampled plots from an $F_{2.3}$ (119–5 sub-okra×MD51ne) population were used to isolate DNA for genetic mapping.

Probe construction and RFLP analysis

A cDNA library was developed from leaf tissue (*C*) of six different cotton cultivars and from fiber tissue (*F*), generating a set of probes using the pGem-11zt(–) vector (Promega) at Biogenetic Service, Inc. from Brookings, South Dakota. Total RNA was isolated according to the methods of MacDonald et al. (1987). The Aviv and Leder (1972) method was used to isolate and purify poly(A)-RNA. The method of Gubler and Hoffman (1983) was used to synthesize cDNA (ds-cDNA). The techniques of Hanahan (1983) were employed for ligation and transformation with *EcoRI* adaptors at the 5′ ends of the ds-cDNA. Multiple copies of the probe were generated via PCR (Saki et al. 1988). The radiolabelling of the probes was done by random priming (Feinberg and Vogelstein 1983). The given references of methodology were slightly modified or adapted for use by Biogenetic Services Inc. (unpublished). Information concerning the availability of the RFLP probes used in this study may be obtained directly from Biogenetic Services, Inc.

RFLP analyses were conducted using bulk samples of juvenile leaf tissue from individual $F_{2.3}$ family single row plots. Pop 1 was assayed using 129 probe/enzyme (*Eco*RI and *Eco*RV) combinations (Shappley et al. 1998). Pop 2 was assayed using 106 probe/enzyme (*Eco*RI and *Eco*RV) combinations (Ulloa and Meredith 2000). Pop 3 was assayed using 94 probe/enzyme (*Eco*RI and *Eco*RV) combinations. A co-dominant pollen color marker also was scored in this population. Pop 4 was assayed using 63 probe/enzyme (*Eco*RI and *Eco*RV) combinations. A co-dominant marker for sub-okra leaf shape was also scored in this population.

A total of 376 probe/enzyme (*Eco*RI and *Eco*RV) combinations were employed to assemble the joinmap using the RFLP fragment information from the four populations. RFLP methods given in "Current protocols in molecular biology" (Ausubel 1978) were used with some minor modifications by Biogenetic Services, Inc. (unpublished). Either *Eco*RI or *Eco*RV restriction enzymes were used in the digestion of the DNA sample, depending upon the probe employed.

Screening of RFLP markers

There were three different scenarios used in the mapping processes on these four populations. (1) Mapping was based on the presence

or absence of an individual fragment, because heterozygotes could not always be reliably scored (or differentiated). Only one of the parents had this unique fragment. This type of marker is referenced as a dominant RFLP. (2) Mapping was based on two fragments, one coming from each parent where the heterozygote could be reliably scored. This type of marker is referenced as a co-dominant RFLP. (3) Mapping was based on more then one fragment (allele) that segregates independently from the remainder of the alleles for a particular locus. If fragments were allelic, all individuals in the mapping population must have at least one of the two fragments. This assumption was merely observational; no allelic test was performed in these populations.

Genetic linkage analysis and map construction

The following procedures were utilized to construct the linkage maps for Pop 2, Pop 3, and Pop 4. Informative bands were scored as present (+) or absent (–) for a dominant marker (expected genotypic ratios 3:1), and if alleles from both parents were identified then the marker was scored as co-dominant (expected genotypic ratios 1:2:1). The JoinMap (Stam and Van Ooijen 1995) computer program was used to test the chi-square goodness-of-fit for expected versus observed genotypic ratios and to develop the final positional maps. Mapmaker/Exp 3.0 (Lander et al. 1987) was also used to develop these genetic maps. LOD scores of 3 to 6 were examined, using the Kosambi map function, and a maximum distance of 40 cM was employed to determine the linkage between two markers. A LOD score of 4.0 was selected to develop the linkage maps. The linkage map for Pop 1 was constructed using MAPMAKER/Exp 3.0 with a LOD score of 3.0 or greater and a genetic distance of 50 cM (Shappley et al. 1998).

The JoinMap (Stam and Van Ooijen 1995) computer program was employed to assemble the four maps, using the modules JMREC for recombination estimation, JMHET for heterogeneity testing, JMPWG for linkage group assignment and for merging linkage data obtained from several populations, and finally JMMAP for map construction. A LOD score of 4.0 was selected to develop the genetic linkage joinmap.

Results

Marker segregation

A total of 129 cDNA probes revealed 138 RFLP loci in the first population (Pop 1). The informative fragments hybridized to 96 DNA samples obtained from bulk-sampled progenies from an $F_{2.3}$ (HS46×MAR) population. Segregation distortion was observed at four RFLP loci. Chi-square values ranged from 0.0 to 29.4. Five of eight co-dominant loci with an abnormal segregation showed an excess of heterozygotes, or an excess of homozygotes, for alleles from the MARCABUCAG8US-1–88 parent (Shappley et al. 1998).

A total of 106 cDNA probes revealed 113 RFLP loci in the second population (Pop 2). The informative fragments hybridized to 119 DNA samples obtained from bulk-sampled progenies from an $F_{2.3}$ (MD5678× Prema) population. Segregation distortion was observed at some RFLP loci with chi-square values ranging from 0.0 to 49.6. Eleven of the 16 loci with the greatest distortion (χ^2>25.0) had greater than expected allelic frequencies from the Prema parent (Ulloa and Meredith 2000).

A total of 94 cDNA probes revealed 100 RFLP loci in the third population (Pop 3). A single morphological marker, pollen color (P_1), gave a total of 101 marker loci. The informative fragments hybridized to 199 DNA samples obtained from bulk-sampled progenies from an $F_{2.3}$ (HQ95–6×MD51ne) population. Segregation distortion was observed at 16 RFLP loci. Chi-square values ranged from 0.0 to 42.4. Among the 16 markers exhibiting segregation distortion, four had an excess of homozygotes represented by the HQ95–6 parent, four had an excess of heterozygosity, and eight had an excess of the alleles (fragments) represented by the MD51ne parent genotype (Table 1).

A total of 63 cDNA probes revealed 69 RFLP loci in the fourth population (Pop 4). In addition a leaf-shape marker was also scored making a total of 70 loci. The informative fragments hybridized to 155 DNA samples obtained from bulk-sampled progenies from an $F_{2.3}$ (119–5 sub-okra×MD51ne) population. Segregation distortion was observed at 15 RFLP loci. Chi-square values ranged from 0.0 to 34.1 among the 15 markers exhibiting segregation distortion; six exhibited an allelic excess from parent 119–5 sub-okra, two from the heterozygote, and seven from the MD51ne. However, observed genotypic

Table 1 Information summary from the four cotton, *G. hirsutum* L., mapping populations used in this study. Genetic linkage maps were developed with a LOD score of 4.0 by the JoinMap computer program (Stam and Van Ooijen 1995)

Mapping population	No. of families	CDNAs[a]	RFLP loci[b]	Linked loci	Unlinked loci	Codom/ dom	Linkage groups	Percentage[c]	Segregation distortion		
									Male parent	Heterozygote	Female parent
HS46×MAR[d]	96	129	138	120	18	84/54	31	18.6	5	3	4
MD5678×Prema[e]	119	90	97	81	16	36/61	17	15.0	11		5
HQ95–6×MD51ne	199	94	100	82	18	59/41	24	18.0	8	4	4
119–5 sub-okra×MD51ne	155	63	69	56	13	28/51	16	11.0	7	2	6
Genetic Joinmap	569	376	404	283	121	138/145	47	31.0	NA	NA	NA

[a] Number of cDNA probes used in each mapping population
[b] Total number of informative alleles (DNA fragments) scored in each population
[c] The approximate percentage of coverage of the total recombinational length of the cotton genome
[d] Mapping population previously reported by Shappley et al. (1998)
[e] Mapping population previously reported by Ulloa and Meredith (2000)
Segregation distortion for RFLP loci, observed with a chi-square greater than 5.0

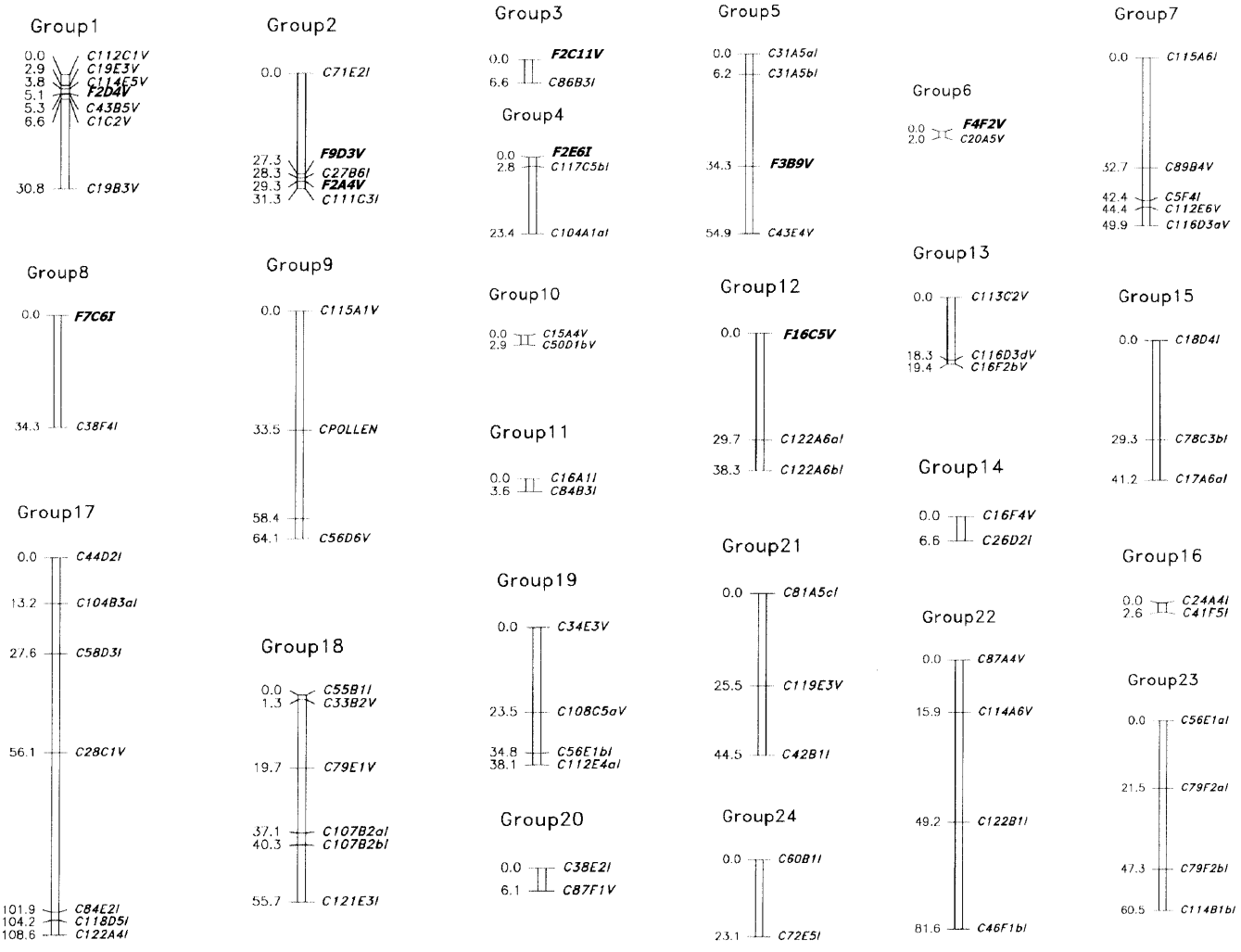


Fig. 1 Cotton RFLP linkage map from 199 bulk-sampled plots of an $F_{2,3}$ ('HQ95–6'×'MD51ne') population (Pop 3) with 82 RFLP loci and the pollen color (P_1) locus (*CPOLLEN*), with 24 linkage groups, covering 830.1 centiMorgans (cM). Map distances between adjacent markers are in cM. The map was constructed by using the JoinMap (Stam and Van Ooijen 1995) computer program, with the Kosambi function and a LOD ratio of 4.0

segregation ratios for six presumably dominant (3:1) markers, revealed a (1:3) genotypic ratio (Table 1). The reason for this result is not known.

Cotton genetic linkage maps

The majority of the RFLP markers used to construct the linkage maps showed normal Mendelian segregation. Table 1 presents a summary from the different genetic linkage maps that were constructed. A large number of progeny (569) was assayed with cDNA probes by *Eco*RI and *Eco*RV enzyme combinations. The number of RFLP linked loci in these maps ranged from 56 to 283 and the number of linkage groups from 16 to 47. The average distance between two markers ranged from 5.3 to 10.0 cM. The map for Pop 1 includes 120 loci covering 865 cM based on the MAPMAKER/Exp 3.0 (Lander et al. 1987) analysis. The 120 loci in this map covered approximately 18.6% of the total recombinational length of the cotton genome (Shappley et al. 1998). The map for Pop 2 includes 81 loci covering 700.7 cM. The 81 loci in our map covered approximately 15% of the total recombinational length of the cotton genome (Ulloa and Meredith 2000). The map for Pop 3 includes 83 loci covering 830.1 cM. The 83 loci in our map covered approximately 18% of the total recombinational length of the genome. The pollen color-marker was found to be linked to three RFLP loci on linkage group 9 (Fig. 1 and Table 1). The map for Pop 4 includes 56 loci covering 520.4 cM based on the JoinMap computer program (Stam and Van Ooijen 1995). The 56 loci in our map covered approximately 11% of the total recombinational length of the cotton genome. The leaf-shape marker was independent (unlinked) of the RFLP markers mapped in this population (Fig. 2 and Table 1).

The joinmap includes 283 loci plus one morphological marker (P_1), making a total of 284 loci that were found to be linked with a LOD of 4.0. Among the 284 loci, 138 segregated as expected with codominant alleles and 145 segregated as expected with dominant alleles.

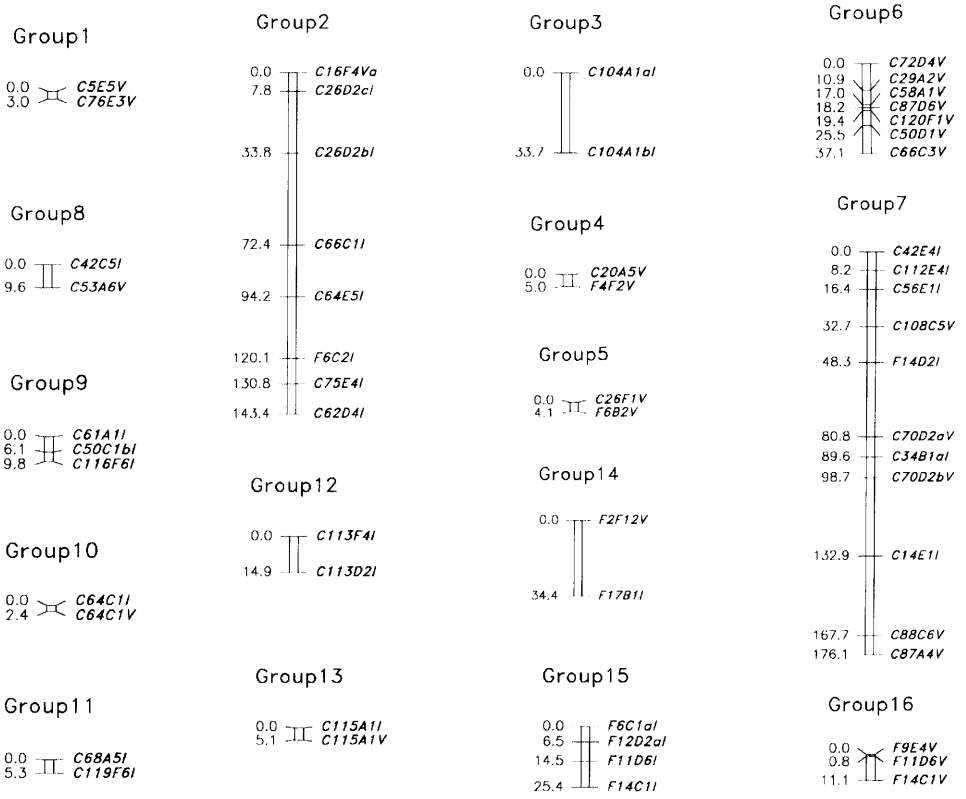


Fig. 2 Cotton RFLP linkage map from 155 bulk-sampled plots of an $F_{2:3}$ ('119–5' Sup-okra×MD51ne) population (Pop 4) with 56 RFLP loci and 16 linkage groups, covering 520.4 centiMorgans (cM). Map distances between adjacent markers are in cM. The map was constructed by using the JoinMap (Stam and Van Ooijen 1995) computer program, with the Kosambi function and a LOD ratio of 4.0

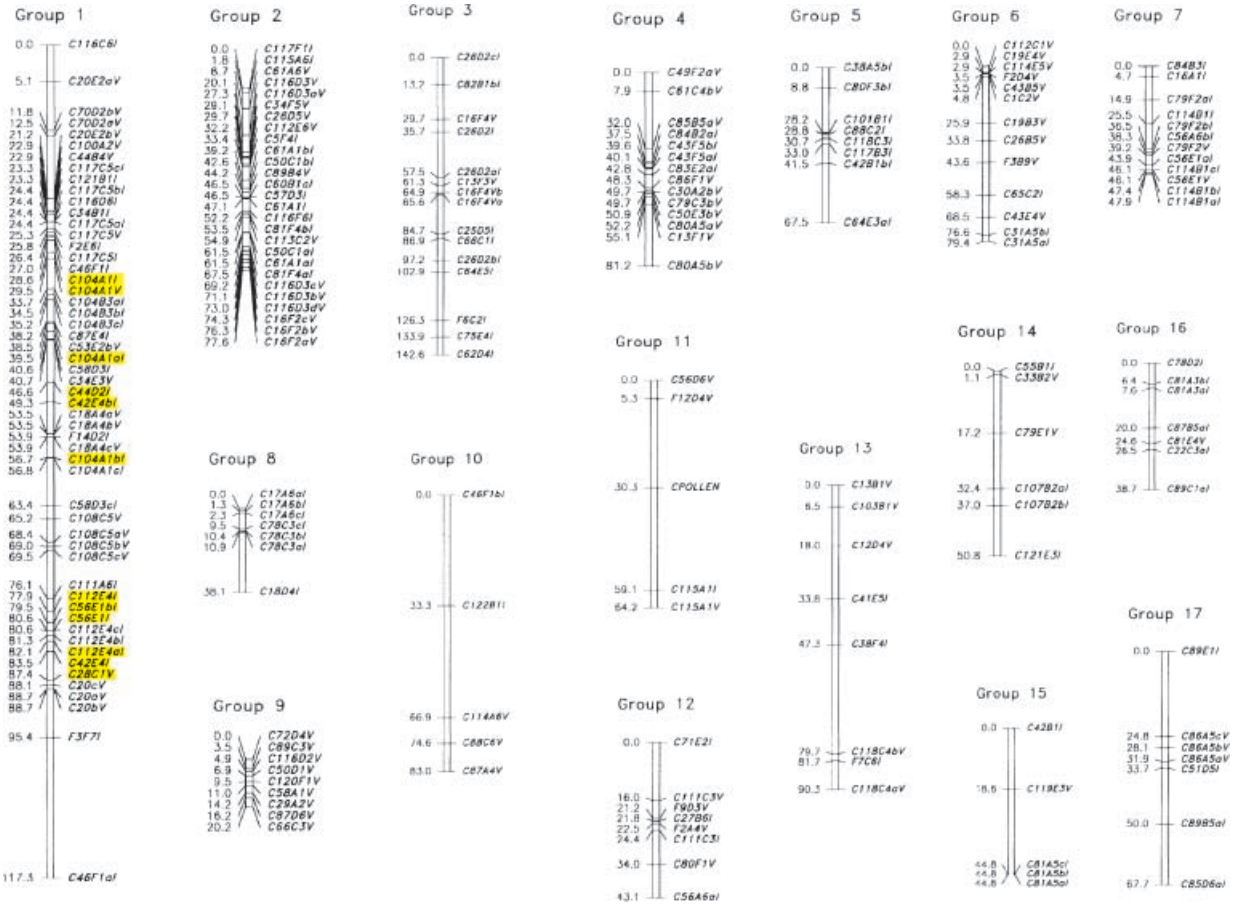
Table 2 Comparison information summary of cDNA, RFLP loci, and the percentage of shared RFLP loci between populations from the four *G. hirsutum* mapping populations

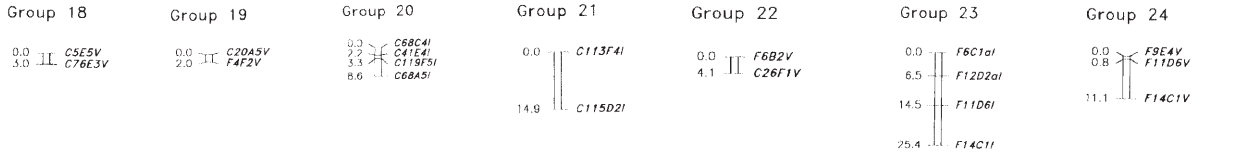
Map comparison	No. of families	I.D.	cDNAs[a]	RFLP loci[b]	Percentage[c]
(HS46×MAR[d]) vs (MD5678×Prema[e])	96 vs 119	Pop 1 vs Pop 2	16	18	16.2
(HS46×MAR) vs (HQ95–6×MD51ne)	96 vs 199	Pop 1 vs Pop 3	44	46	41.0
(HS46×MAR) vs (119–5 sub-okra×MD51ne)	96 vs 155	Pop 1 vs Pop 4	15	18	16.2
(MD5678×Prema) vs (HQ95–6×MD51ne)	119 vs 199	Pop 2 vs Pop 3	11	11	9.9
(MD5678×Prema) vs (119–5 sub-okra×MD51ne)	119 vs 155	Pop 2 vs Pop 4	10	10	9.0
(HQ95–6×MD51ne) vs (119–5 sub-okra×MD51ne)	199 vs 155	Pop 3 vs Pop 4	10	10	9.0

[a] Number of cDNA probes that hybridized in two populations (shared RFLP clones)
[b] Number of informative alleles (DNA fragments) scored and hybridized in two populations (shared RFLP loci)
[c] The percentage of shared RFLP loci between two populations from a total of 111 shared RFLP loci from the four populations
[d] Mapping population previously reported by Shappley et al. (1998)
[e] Mapping population previously reported by Ulloa and Meredith (2000)

The 284 loci covered 1,502.6 cM based on the JoinMap computer program (Stam and Van Ooijen 1995). Thus, the 284 loci covered approximately 31% of the total recombinational length of the cotton genome (Fig. 3 and Table 1). Twenty one linkage groups contained only two markers per group (Fig. 3). More markers are needed to accurately assign these smaller groups. Segregation distortion was observed at 59 RFLP loci. Chi-square values

Fig. 3 Cotton RFLP genetic-linkage joinmap from bulk-sampled plots of four $F_{2:3}$ cotton populations, two previously reported (Shappley et al. 1998; Ulloa and Meredith 2000), with 283 RFLP loci and the pollen color (P_1) locus, with 47 linkage groups, covering 1,502.6 centiMorgans (cM). Map distances between adjacent markers are in cM. The map was constructed by using the JoinMap (Stam and Van Ooijen 1995) computer program, with the Kosambi function and a LOD ratio of 4.0 ▶


Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
Group 8
Group 9
Group 10
Group 11
Group 12
Group 13
Group 14
Group 15
Group 16
Group 17


Group 18
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Group 21
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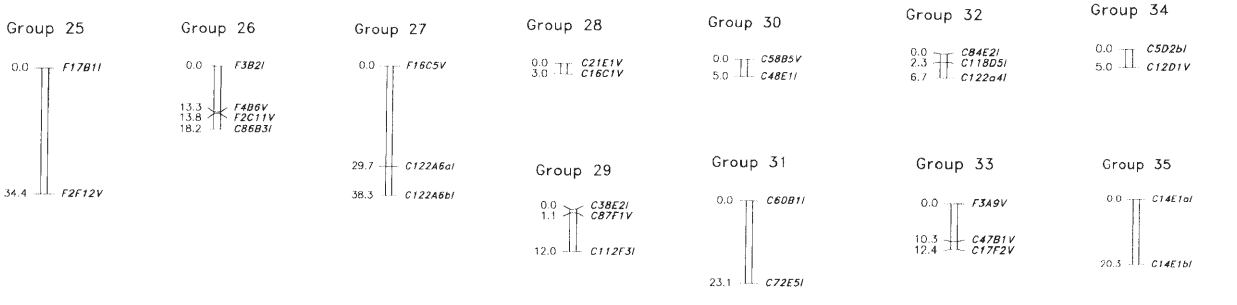
Group 23
Group 24


Group 25
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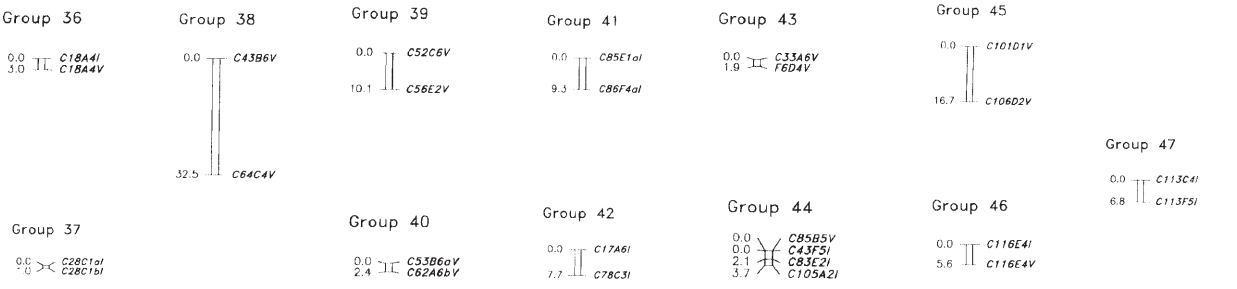
Group 35


Group 36
Group 37
Group 38
Group 39
Group 40
Group 41
Group 42
Group 43
Group 44
Group 45
Group 46
Group 47

ranged from 0.0 to 49.6. Among the 59 markers exhibiting segregation distortion, 31 had an excess of homozygotes represented by the male parent, nine had an excess of heterozygosity, and 19 had an excess of the alleles (fragments) represented by the female parent genotype. Segregation distortion from Mendelian segregation ratios was observed up to 21.0% (Table 1).

RFLP loci comparison between populations

The complete scored molecular data set of the four populations was used to determine the homology of RFLP loci. A core of 104 cDNA probes was common between populations, yielding a total of 111 common RFLP loci.

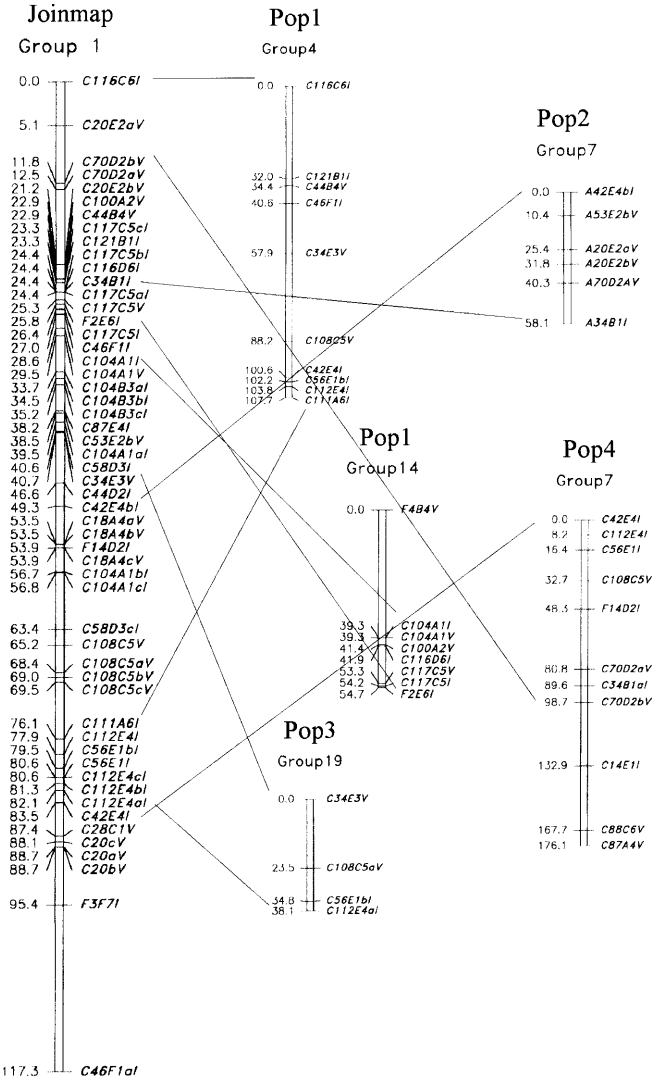


Fig. 4 Amalgamation of the joinmap linkage group No 1 as compared to groups Nos. 4 and 14 from the first population (Pop 1), group No. 7 from the second population (Pop 2), group No. 19 from the third population (Pop 3), and group No. 7 from the fourth population (Pop 4) to further investigate gene (RFLP loci) order. The joinmap linkage group No. 1 was constructed by using the JoinMap (Stam and Van Ooijen 1995) computer program, with the Kosambi function and a LOD ratio of 4.0

Table 2 presents a summary of the number of cDNA probes as well as RFLP loci that were common among populations. The maps from Pop 1 and Pop 3 were the most similar with 41.0% shared heterozygous RFLP loci (Table 2). They shared 44 cDNA probes and 46 RFLP loci. The RFLP loci involved at least 19 linkage groups. Fourteen of the groups had at least two linked loci in the two populations. The maps for Pop 2 and Pop 4 were the least similar. They shared ten cDNA probes and ten RFLP loci. Four linkage groups had at least two loci linked in the two populations (Table 2). MD5678 and 1195–5 evolved from the DES program and shared similar pedigrees, while Prema and MD51ne are very different agronomically. Based on 111 shared RFLP loci, the percentage of common heterozygous loci between populations varied from 9.0% to 41.0%. Identification of homologous RFLP loci in a linkage group reveals the approximate number of loci of cDNA-probes monomorphic in one population but polymorphic in the other. Knowledge of homology represents a means to greatly increase the informativeness of the genetic joinmap in the tetraploid *G. hirsutum* (Figs. 3 and 4). The cDNA probes used to construct the joinmap detected multiple segregating loci. This or a higher level of segregation has been observed in previous studies (Bernatzky and Tanksley 1986; Kianian and Quiros 1992).

At least five linkage groups from the four cotton populations blended into the largest joinmap linkage group, group No. 1 (Fig. 4). In the joinmap linkage group No. 1, the order of RFLP loci changed moderately or drastically depending on the locus. Characterization of the comparative organization of heterozygous RFLP loci in linkage groups from the different populations promises to increase the marker density of the molecular upland cotton joinmap and to facilitate both genetic and physical mapping applications.

Discussion

An RFLP joinmap of *G. hirsutum* has been established as a first step toward further understanding its structural genome.

Sixteen percent of the RFLP loci showed segregation distortion among the four populations. Analyses of the parental origin of each RFLP allele revealed slight preference for the MAR, Prema, and MD51ne genotypes in three of the four populations. Segregation distortion also has been reported in other crops such as maize (Rhoades 1941; Robertson 1984), tomato (Paterson et al. 1991), and *Brassica* (Kianian and Quiros 1992). Kianian and Quiros (1992) compared individual genetic maps and found that distortion in segregation ratios increased with the level of divergence of the parents. They attributed the distortion to the differentiation of parental chromosomes. Occasionally such preferential segregation can be used as a basis for genotypic selection among individuals, accelerating introgression of a desired chromosomal segment into a new genetic background (Young and

Tanksley 1989). The $F_{2.3}$ cotton populations were the result of artificial selection and breeding. The distorted segregation RFLP loci observed in these mapping populations may be due to the complex breeding pedigree of the parents used. At least one of the parents in each mapping population originated from an introgression of a triple hybrid as well as the introgression of segments of DNA from *G. barbadense*, which is quite common in Upland cotton.

The joinmap and the comparison of linkage groups from the different populations (Figs. 3 and 4, and Table 2) provide information concerning linkage relationships among RFLP loci. This information also increases our understanding of genetic mapping (structural genomics) for Upland cotton. Recombination in *G. hirsutum* may occur in blocks only on certain chromosomes. In an allotetraploid such as cotton, two genomes are in the same nucleus. Therefore, recombination rates might be suspected to be affected by life-history, population genetics, and ecological covariables (Brubaker et al. 1999). Chromosome pairing is often disturbed at meioses in synthetic allopolyploids. Evidence of locus duplication or deletion as a consequence of polyploidization events may contribute to changes in recombination rates (Small and Wendel 2000).

This is the first genetic linkage joinmap assembled in *G. hirsutum* with a core of RFLP markers assayed on different cotton populations with different genetic backgrounds (Acala, Delta, and Texas plain). It is evident that mapping at the multi-population level has many advantages over that based on a single population. Gene order and map distances are estimated more accurately with a large number of mapped loci in different populations with different genetic backgrounds. The RFLP loci mapped in this study will be used to identify QTLs for agronomic, physiological, and fiber quality traits, as well as for the identification of RFLP loci lineage for *G. hirsutum* from its diploid progenitors (the A and D genomes).

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References

Arumunganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol 9:208–218

Ausubel F (ed) (1978) Current protocols in molecular biology. Green Publishing Company, New York

Aviv H, Leder P (1972) Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA 69:1408–1412

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Bridge RR (1986) Registration of 'DES 119' cotton. Crop Sci 26:646–647

Brubaker CL, Paterson AH, Wendel JF (1999) Comparative genetic mapping of allotetraploid cotton and its diploid progenitors. Genome 42:184–203

Brubaker CL, Cantrell R, Giband M, Lyon BR, Wilkins TA (2000) Letter to Journal of Cotton Science Community. J Cotton Sci 4:149–151

Calhoun DS, Bowman DT, May OL (1997) Pedigrees of Upland and Pima cotton cultivars released between 1970 and 1995. Bull 1069, Miss Agr and Forestry Exp Stn, Stoneville, Mississippi

El-Zik KM, Thaxton PM (1990) Registration of 'Tamcot HQ95' cotton. Crop Sci 30:1359–1360

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonucleases fragments to highly specific activity. Anal Biochem 137:266–267

Fryxell PA (1984) Taxonomy and germplasm resources. In: Kohel RJ, Lewis CF (eds) Cotton 1984. Agron Monogr 24, ASA, CSSA, and SSSA Madison, Wisconsin, pp 27–57

Geever RF, Katterman FRH, Endrizzi JE (1989) DNA hybridization analyses of a *Gossypium* allotetraploid and two closely related diploid species. Theor Appl Genet 77:553–559

Giese H, Holm-Jensen AG, Jensen HP, Jensen J (1993) Localization of the *laevigatum* powdery mildew resistance gene to barley chromosome 2 by the use of RFLP markers. Theor Appl Genet 85:897–900

Grant V (1975) Genetics of flowering plants. Columbia University Press, New York

Gubler U, Hoffman BJ (1983) A simple and very effective method for generating cDNA libraries. Gene 25:263–269

Hanahan D (1983) Studies on the trasformation of *Escherichia coli* with plasmids. J Mol Biol 166:557–580

Jiang C-X, Wright RJ, El-Zik KM, Paterson AH (1998) Polyploid formation created unique avenues for response to selection in *Gossypium* (Cotton). Proc Natl Acad Sci USA 95:4419–4424

Jiang C-X, Wright RJ, Woo SS, DelMonte TA, Paterson AH (2000) QTL analysis of leaf morphology in tetraploid *Gossypium* (cotton). Theor Appl Genet 100:409–418

Kianian SF, Quiros CF (1992) Generation of a *Brassica oleracea* composite RFLP map: linkage arrangements among various populations and evolutionary implications. Theor Appl Genet 84:544–554

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lee SH, Bailey MA, Mian MAR, Carter Jr. TE, Ashley DA, Hussey RS, Parrott WA, Boerma HR (1996) Molecular markers associated with soybean plant height, lodging, and maturity across locations. Crop Sci 36:728–735

MacDonald RJ, Swift GH, Przybyla AE, Chirgwin JM (1987) Isolation of RNA using guanidinium salts. Methods Enzymol 152:219–223

Meredith Jr WR (1993) Registration of 'MD51ne' cotton. Crop Sci 33:1415

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparision across species, generations, and environments. Genetics 127: 181–197

Paterson AH, Lander ES, Hewitt JD, Paterson S, Lincoln SE, Tanksley SD (1998) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Reinisch AJ, Dong JM, Brubaker CL, Stelly DM, Wendel JF, Paterson AH (1994) A detailed RFLP map of cotton, *Gossypium hirsutum*×*Gossypium barbadense* : chromosome organization and evolution in a disomic polyploid genome. Genetics 138:829–847

Rhoades MM (1941) Different rates of crossing over in male and female gametes of maize. J Am Soc Agron 33:603–615

Robertson DS (1984) Different frequency in the recovery of crossover products from male and female in maize. Genetics 107: 117–130

Saki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:47–491

Shappley ZW, Jenkins JN, Meredith Jr WR, McCarty JC (1998) An RFLP linkage map of Upland Cotton, *Gossypium hirsutum* L. Theor Appl Genet 97:756–761

Slocum MK, Figdore SS, Kennard WC, Suzuki JY, Osborn TC (1990) Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*. Theor Appl Genet 80:57–64

Small RL, Wendel JF (2000) Copy number lability and evolutionary dynamics of the *Adh* gene family in diploid and tetraploid cotton (*Gossypium*). Genetics 155:1913–1926

Stam P, Van Ooijen JW (1995) JoinMap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen, The Netherlands

Stephens JC, Miller FR, Rosenow DT (1967) Conversion of alien *Sorghum* to early combine genotypes. Crop Sci 7:396

Ulloa M, Cantrell RG (1998) QTL Analysis in cotton. In: Proc Int Plant and Animal Genome Conf VI. San Diego, California, 18–22 Jan 1998. Scherago Int, New York, pp 351 (Abs)

Ulloa M, Cantrell RG, Percy R, Lu Z, Zeiger E (2000). QTL analysis of stomatal conductance and relationship to lint yield in an interspecific cotton. J Cotton Sci 4:10–18

Ulloa M, Meredith Jr WR (2000) Genetic linkage map and QTL analysis of agronomic and fiber quality traits in an intraspecific population. J Cotton Sci 4:161–170

Wendel JF, Brubaker CL, Percival E (1992) Genetic diversity in *Gossypium hirsutum* and the origin of Upland cotton. Am J Bot 79:1291–1310

Young ND, Tanksley SD (1989) Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor Appl Genet 77:95–101

Yu J, Yong-Ha P, Lazo GR, Kohel RJ (1998) Molecular mapping of the cotton genome: QTL analysis of fiber quality properties. In: Proc Beltwide Cotton Conf, San Diego, California, 5–9 Jan 1998. Natl Cotton Counc Am, Memphis, Tennesee, pp 485 (Abs)