D-Subgenome Bias of *Xcm* Resistance Genes in Tetraploid Gossypium (Cotton) Suggests That Polyploid Formation Has Created Novel Avenues for Evolution

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Manuscript received November 19, 1997
Accepted for publication April 15, 1998

ABSTRACT

A detailed RFLP map was used to determine the chromosomal locations and subgenomic distributions of cotton (Gossypium) genes/QTLs that confer resistance to the bacterial blight pathogen, *Xanthomonas campestris* pv. *malvacearum* (*Xcm*). Genetic mapping generally corroborated classic predictions regarding the number and dosage effects of genes conferring *Xcm* resistance. One recessive allele (b_6) was a noteworthy exception to the genetic dominance of most plant resistance alleles. This recessive allele appeared to uncover additional QTLs from both resistant and ostensibly susceptible genotypes, some of which corresponded in location to resistance (R)-genes effective against other *Xcm* races. One putatively "defeated" resistance allele (B_3) reduced severity of *Xcm* damage by "virulent" races. Among the six resistance genes derived from tetraploid cottons, five (83%) mapped to D-subgenome chromosomes—if each subgenome were equally likely to evolve new R-gene alleles, this level of bias would occur in only about 1.6% of cases. Possible explanations of this bias include biogeographic factors, differences in evolutionary rates between subgenomes, gene conversion or other intergenomic exchanges that escaped detection by genetic mapping, or other factors. A significant D-subgenome bias of *Xcm* resistance genes may suggest that polyploid formation has offered novel avenues for phenotypic response to selection.

IN contrast to important diploid botanical models such as Arabidopsis, rice, or tomato, the polyploidy of cultivated cotton adds additional dimensions to host-pathogen interactions and other traits. The merger of divergent genomes in a common nucleus has been variously argued to represent "a shift from genetic flexibility to genotypic fixation" (*cf.* Mackey 1970; Stebbins 1971) and "an opportunity for novel avenues of response to selection" (*cf.* Levin 1983). Cotton is a disomic allotetraploid comprised of two genomes that form bivalents at meiosis. Polyploid cotton arose about 1–2 million years ago as a result of interspecific hybridization between an Old World A-genome diploid taxon and a New World D-genome diploid taxon (Beasley 1940; Wendel 1989).

Bacterial blight of cotton, incited by the pathogen *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye (*Xcm*), is a classic example of a plant-pathogen relationship in an important crop. A 50-year history of research has led to our current understanding of both plant resistance of cotton and pathogenicity of the bacterium. Key steps in dissecting the cotton-*Xcm* interaction have included the identification of the pathogenic organism (*Xcm*) (Dye *et al.* 1980), host differentials (Hunter *et al.* 1968; Brinkerhoff 1970), pathogen races with differential virulence (Brinkerhoff 1963, 1970; Cross 1963; Follin 1983; Verma 1986), and plant germplasm that confers resistance to various races of the pathogen (Knight and Clouston 1939, 1941; Knight 1944, 1953; El-Zik 1967; El-Zik and Bird 1970; Bird 1986; Wallace and El-Zik 1989, 1990). Advancements at the cellular level have led to a better understanding of the disease cycle (Jakkanwar and Bhagwat 1971; Verma 1986), plant defense responses (Al-Mousawi *et al.* 1983) and metabolites (Essenberg *et al.* 1982) that may function in pathogenicity or plant defense.

Analysis of the subgenomic (A vs. D) distribution of genes conferring *Xcm* resistance in tetraploid AD-genome cottons provides an interesting system for studying the impact of allopolyploid formation on host-pathogen interactions. A-genome Gossypium taxa show near-immunity to *Xcm*, which was deemed sufficiently valuable to impel introgression into cultivated tetraploids (Knight 1953). The *Xcm* immunity of A-genome cottons is consistent with the probable Old World origin of the pathogen (Knight 1948b; Knight and Hutchinson 1950), and the observation that new virulent strains appear to have arisen in Africa (Follin 1981, 1983). Although D-genome diploids show varying degrees of resistance, none show "immunity" (Knight 1948b), and they have not been an important source of resistance genes for the improvement of cultivated cottons. A total of 19 races of *Xcm* pathogenic to cotton

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are currently recognized in the U.S. (Hunter *et al.* 1968; Verma 1986) and additional virulent isolates of the pathogen have appeared in Africa (Follin 1981, 1983).

The specific objectives of this study were to use an established RFLP map (Reinisch *et al.* 1994) to identify and map genes which confer resistance to several races of *Xcm*, to shed light on the subgenomic distribution of *Xcm* resistance alleles that evolved subsequent to polyploid formation in cotton, and to provide diagnostic DNA markers for use in cotton improvement. We mapped the B_2, B_3, b_6, and B_{12} genes in cotton, four of the most important genes that confer resistance to *Xcm* among the 22 reported to date (Brinkerhoff 1970; Verma 1986; Hillocks 1992). A significant D-subgenome bias of *Xcm* resistance genes may suggest that polyploid formation has offered novel avenues for phenotypic response to selection in that the genome formerly not exposed to the pathogen is the source of new resistance genes.

MATERIALS AND METHODS

Mating and field plot design: Four F_2-selfed populations were developed from crosses between each of four different resistant *Gossypium hirsutum* parents and a single *Gossypium barbadense* parent, "Pima S-7," that is highly susceptible to all *Xcm* races. Three of the *G. hirsutum* parents were developed by backcrossing the B_2, B_3, and b_6 resistance phenotypes from R. L. Knight's BAR Sakel lines (Knight and Clouston 1939; Knight 1946, 1955) into the Empire 8-0-8 genotype (Bird 1960). These putatively nearly-isogenic lines are designated Empire B2, Empire B3, and Empire B2b6. The fourth *G. hirsutum* parent, "S295" (Girardot *et al.* 1986), contains the B_{12} resistance gene, which confers a high level of resistance to all *Xcm* races presently found in the U.S. and also many races which have recently evolved in Africa (Wallace and El-Zik 1989, 1990). We refer to each segregating population by its *G. hirsutum* parent.

In the summer of 1995, 119 to 150 F_2 (self-pollinated progeny of an F_1 hybrid between homozygous parents) individuals of each of the four populations were grown in the field at College Station, Texas. Each F_2 population was planted in a completely randomized design with plants spaced 62 cm apart on rows 1 m wide. Seedlings were started in pellets and transplanted to the field to insure better germination and survival. Standard irrigation, cultural, and pest management practices were applied throughout this study.

Phenotyping: The fifth true leaf of each F_2 plant was inoculated at separate sites with *Xcm* Races 2, 4, 7, and 18 at a concentration of approximately 1×10^6 bacteria per ml using the toothpick method (Bird 1982; Thaxton and El-Zik 1993). Ten days after inoculation, disease reactions were scored on a scale of 1 (highly resistant) to 10 (highly susceptible) (Thaxton and El-Zik 1993).

RFLP genotyping: Selective genotyping (Lander and Botstein 1989) of 28 resistant and 28 susceptible individuals from each population was used to identify genomic regions tentatively associated with resistance. In the Empire B2, Empire B3, and S295 populations, selection of extreme individuals was based on reaction to *Xcm* Race 2. In the Empire B2b6 population, selections were based on reaction to *Xcm* Race 18 (targeting the b_6 gene). In no case did the standard deviation of the disease score for a subgroup (resistant *vs.* susceptible) overlap the population mean, or the mean of the opposing subgroup (Table 1). Lander and Botstein (1989) estimated that progeny with phenotypes more than 1 standard deviation from the population mean contribute about 81% of the total linkage information in a population.

Genomic DNA was extracted from 4 g of young leaves collected from individual F_2 plants as described (Paterson *et al.* 1993). RFLP analysis was performed as described (Reinisch *et al.* 1994) using 226 RFLP markers (from Reinisch *et al.* 1994) spaced at approximately 20-cM intervals throughout the cotton genome.

Data analyses: *Trait means, histograms, correlations, broad sense heritability, and chi-square analyses:* Histograms of *Xcm* reaction were made for each population and correlations among traits (Figure 1) were calculated using QGene (version 2.26; Nelson 1997), using the R-trait correlations function.

For qualitative analyses, a disease grade of <6 was used as the threshold to classify individuals as resistant (Table 2). Segregation for *Xcm* resistance in the Empire B2, Empire B3, and S295 populations was tested against a single locus model. In the Empire B2b6 population, a two locus model (one dominant, one recessive) was used to test segregation for resistance to *Xcm* Race 2, and a recessive model was used to test segregation for resistance to *Xcm* Race 18.

Linkage maps and QTL analysis: Linkage maps were constructed for each population using MapMaker (Lander *et al.* 1987), as previously described (Reinisch *et al.* 1994). Resistance phenotypes were mapped as discrete genetic loci (based on the stated disease grade threshold of <6) and compared to the locations inferred from quantitative trait loci (QTL) interval analysis (Lander and Botstein 1989) of quantitative disease grades (as described above). Discrepancy may suggest possible "escapes" in phenotyping, or the presence of modifier genes. A LOD threshold of 3.0 was used to infer the presence of QTLs in the recombinationally large genome of cotton to assure that the likelihood of even a single false positive in each population remained below 5%. In each population, the largest gene/QTL found for each trait was "fixed," then the genome was rescanned to test for the presence of additional QTLs with smaller effects (Lin *et al.* 1995). This "fix and rescan" procedure was repeated until no additional QTLs were found. The gene action of each QTL (additive, dominant, or recessive) was tested as described (Paterson *et al.* 1991). The types of gene action indicated for QTLs are those that could not be rejected by 1-LOD or more as unlikely.

RESULTS

Phenotypic variation for *Xcm* reactions: *Empire B2 population:* Individual plant reactions to *Xcm* Races 2 and 4 were highly correlated ($r = 0.99$; Figure 1b), clearly bimodal, with a broad-sense heritability of 0.91 (Figure 1a), and did not deviate significantly from a single-gene model ($P = 0.30$; Table 2), consistent with published data (Knight 1953; Innes 1983). All plants were highly susceptible to *Xcm* Races 7 and 18.

Empire B3 population: Individual plant reactions to Races 2 and 4 were highly correlated ($r = 0.73$; Figure 1f), continuously distributed from resistant to susceptible, with a broad-sense heritability of 0.79 (Figure 1e), and deviated significantly from a single-gene dominant model ($P = 1.4 \times 10^{-22}$; Table 2). Incompletely dominant gene action of B_3 has been reported (Innes 1983), and would account for both the observed deviation, and

TABLE 1

Average disease grades of cotton subpopulations used for selective genotyping

	Empire B2	Empire B3b6[a]		Empire B3		S295	
	Race 2	Race 2	Race 18	Race 2	Race 18	Race 2	Race 18
Resistant	2.00 ± 0	1.96 ± 0.64	1.89 ± 0.74	3.86 ± 1.33	7.71 ± 0.66	1.00 ± 0	1.00 ± 0
Susceptible	9.21 ± 0.42	9.18 ± 0.98	9.61 ± 0.57	8.86 ± 0.59	9.11 ± 0.31	9.57 ± 0.50	9.46 ± 0.58
Overall	3.59 ± 2.91	2.70 ± 2.10	6.88 ± 3.19	6.32 ± 2.38	8.27 ± 0.74	4.31 ± 3.46	4.14 ± 3.47

Each resistant or susceptible subpopulation was composed of 28 individuals.

[a] Selected based on Race 18 phenotypes to target the b_6 gene(s); the 56 individuals selected also segregated 45 resistant: 11 susceptible to *Xcm* Race 2, with means and standard deviations shown.

the relatively continuous distribution of phenotypes in this population. Correlations between reactions to Races 2 or 4, with reactions to *Xcm* Races 7 or 18 ($r = 0.5$–0.59; Figure 1, g and h) were much higher than expected, since all individuals showed water-soaked lesions indicating a susceptible reaction to *Xcm* Races 7 and 18. Broad sense heritability for *Xcm* Race 18 of 0.58 (Figure 1h) was also higher than expected.

Empire B2b6 population: Individual plant reactions to Races 2 and 4 were bimodal, highly-correlated ($r = 0.95$; Figure 1j), with a broad-sense heritability of 0.91 (Figure 1i), and deviated from a two-locus model ($P = 4.0 \times 10^{-3}$; Table 2). Individual plant reactions to *Xcm* Races 7 and 18 were bimodal, highly-correlated ($r = 0.99$; Figure 1l), with a broad-sense heritability of 0.95 (Figure 1l), and did not deviate significantly from a single-gene model ($P = 0.12$; Table 2). Resistance to Races 2 and 4 (B_2) was dominant, while resistance to Races 7 and 18 (b_6) was recessive, both consistent with prior observations (Knight 1953; Innes 1983). Significant correlations ($r = 0.28$; Figure 1, k and l) between individual plant reactions to *Xcm* Race 2 (or 4) and Race 18 (or 7) were lower than expected, but consistent with the expectation (Knight 1953; Saunders and Innes 1963) that b_6 confers resistance to all 4 races.

S295 population: Disease reactions to all *Xcm* races were highly correlated ($r = 0.97$–0.99; Figure 1, n–p), bimodal, with a broad-sense heritability of 0.98 (Figure 1m,p), and did not deviate significantly from a single-gene model ($P = 0.06$; Table 2), as expected from the prior assertion (Wallace and El-Zik 1989, 1990) that S295 confers resistance to all these *Xcm* races.

Genome transmission: *Segregation and recombination:* We assembled linkage maps of 162, 224, 253, and 184 loci, in Empire B2, Empire B3, Empire B2b6, and S295, respectively, by using 226 RFLP markers that detected 310 loci at an average spacing of 17.5 cM on the previously published map (Reinisch *et al.* 1994). The maps

TABLE 2

Qualitative analysis of segregation for bacterial blight resistance in four cotton populations

Disease grade	Empire B2 Race 2	Empire B2b6 Race 2	Empire B2b6 Race 18	Empire B3 Race 2	S295 Race 18
1	0	11	9	0	32
2	108	85	14	7	35
3	9	19	13	12	16
4	0	0	2	16	6
5	1	0	1	2	0
6	0	0	0	10	0
7	0	1	2	14	0
8	3	1	19	25	14
9	23	4	48	19	15
10	6	5	18	3	16
Sum	150	126	126	108	134
Model	3:1	13:3	1:3	3:1	3:1
χ^2	1.08	8.3	2.38	95.6	3.59
P value	0.30	4.0×10^{-3}	0.12	1.4×10^{-22}	0.06

Only phenotypes to *Xcm* Races 2 and 18 are included, since Races 2 and 4 reactions, and Races 7 and 18 reactions, were closely correlated. A disease grade of <6 was considered to indicate resistance, and the ratio of resistant to susceptible plants was tested against the predicted genetic model. Phenotypic information to *Xcm* Race 2 was not obtained on 11 individuals in Empire B3, accounting for the discrepancy with total number of individuals.

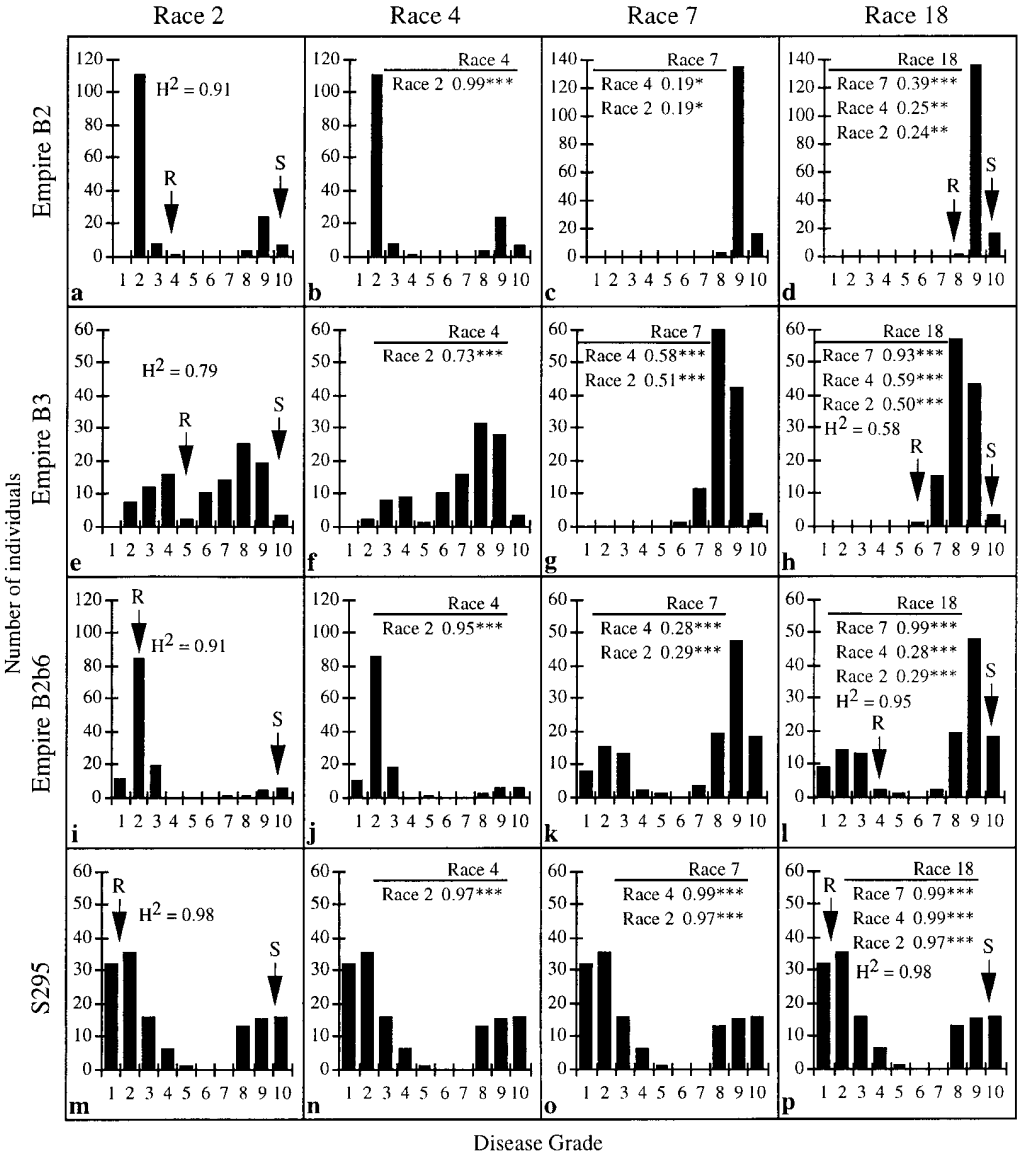

Figure 1.—Frequency distribution of resistance to *Xcm* Races 2, 4, 7, and 18 in the Empire B2, Empire B3, Empire B2b6, and S295 populations. Average values for resistant (R) and susceptible (S) parents are indicated by arrows. Correlations among disease scores for *Xcm* Races 2, 4, 7, and 18 are provided for each population (*, **, *** denote significance at ≤0.05, ≤0.01, and ≤0.001 levels, respectively). H^2 indicates broad-sense heritability, calculated as described (Falconer and Mackay 1996). Disease reactions were scored on a scale of 1 (highly resistant) to 10 (highly susceptible).

included 48, 45, 49, and 48 linkage groups spanning 921.6, 1746.8, 2100.9, and 1266.6 cM with an average distance between linked markers of 5.7, 7.8, 8.3, and 6.9 cM. An additional 59, 49, 64, and 62 loci were unlinked to the maps. Based on the published map, the marker loci segregating in these four crosses are discernibly linked (at 20 cM or less) to 71.5%, 96.2%, 98.9%, and 83.0%, respectively, of the cotton genome.

Genes/QTLs conferring bacterial blight resistance: The chromosomal locations of genes and QTLs associated with each resistance phenotype, based on quantitative measures of disease reaction, are presented in Figure 2. Each resistance phenotype was also mapped as a discrete genetic locus, considering a disease grade of <6 as "resistant." Qualitative analysis assigned resistance loci to the correct linkage group in all cases, but tended to place loci at the ends of the linkage group, or in large gaps between markers (see discussion).

A total of seven QTLs was detected among the four populations. The inheritance of resistance in each population was as follows:

Empire B2 population: A region near the DNA marker *G1219* on D-subgenome chromosome *20* (Figure 2) explained 98.0% (LOD 103.1) of the phenotypic variation in reaction to *Xcm* Races 2 and 4 (Table 3). The GH (*G. hirsutum*) allele was dominant. If scored as a discrete genetic locus, the maximum-likelihood location of this trait was a large gap between markers, about 26 cM from *G1219* (Figure 2). Forcing the discrete genetic locus into the interval containing the likelihood peak inflated the distance between *pAR 335b* and *G1219* by 4.3 cM (from 19.5 to 23.8 cM). Even a low frequency of false-positive or false-negative results in phenotyping would be sufficient to account for the location of the "discrete" gene.

Empire B3 population: A region near the DNA marker *pGH510a* on D-subgenome chromosome *20* accounted for 88.2% (LOD 23.2) of the phenotypic variation in

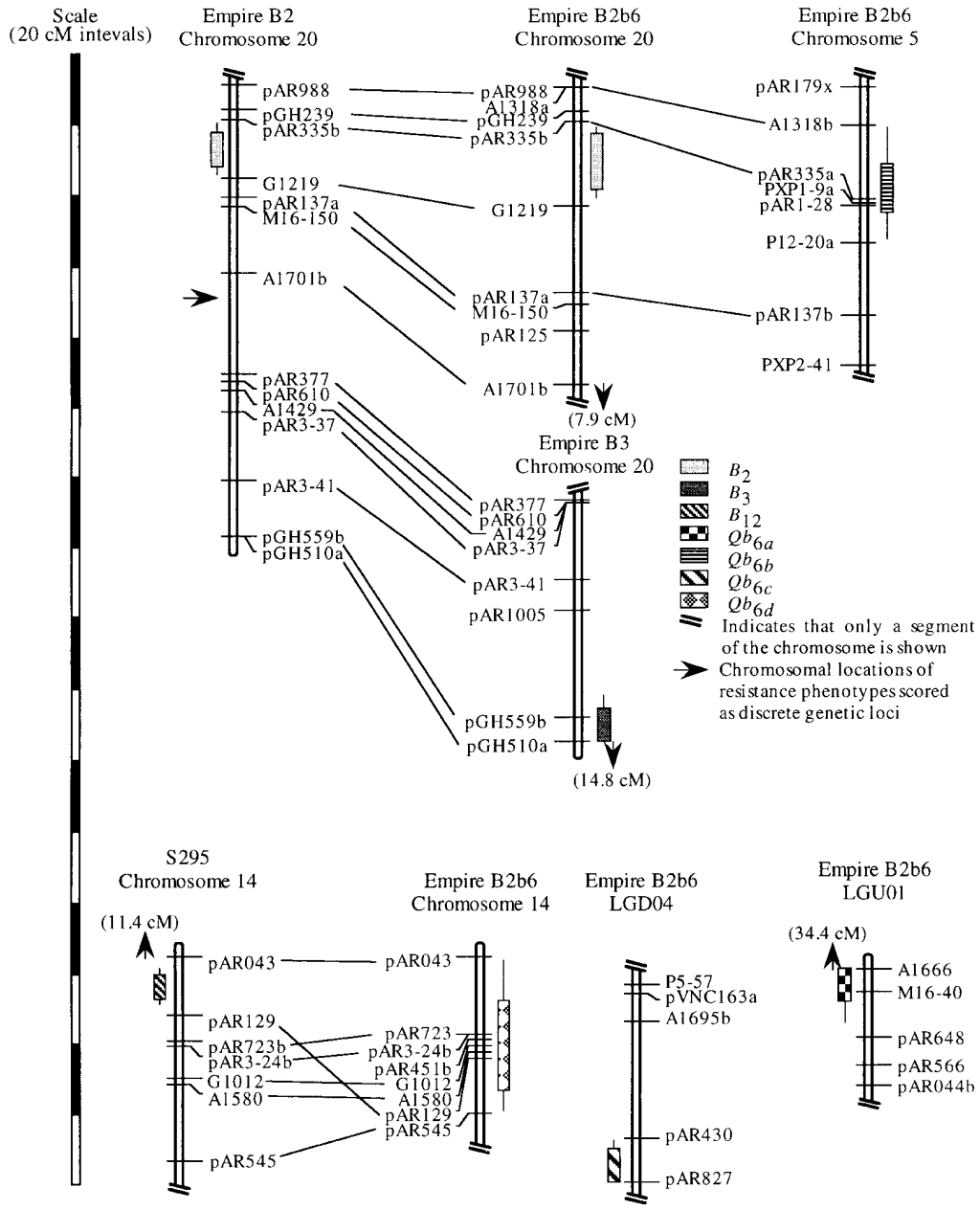

Figure 2.—Chromosomal locations of genes conferring resistance to bacterial blight in cotton. Bars along the linkage groups indicate 90% (1-LOD) likelihood intervals for the QTLs, and whiskers indicate 99% (2-LOD) likelihood intervals. Arrows indicate maximum likelihood locations of genes/QTLs when assessed as discrete phenotypes, and the values near each arrow indicate genetic distance (in centiMorgans) from the end of the linkage group.

reaction to Races 2 and 4 (Table 3). Although B_3 is more than 50 cM away from B_2, the finding that both are on the same chromosome supports classic data suggesting linkage between these genes (Knight 1944). B_3 showed a modest deviation from additivity toward dominance ($d/a = 0.41$), consistent with earlier reports (Innes 1983). Incompletely dominant gene action of B_3 has been reported (Innes 1983), and may account for the observed deviation from single-gene segregation in this population (see above). When scored as a discrete genetic locus, the maximum-likelihood location of B_3 is well outside the QTL likelihood interval, again suggesting the possibility of occasional escapes (Figure 2). Because quantitative analysis mapped B_3 to the end of the chromosome, it was not possible to assess the "inflation" in size of an interval as a result of adding B_3 as a discrete phenotype.

Unexpectedly, B_3 explains 53.4% (LOD 10.56) of phenotypic variation in reaction to *Xcm* Races 7 and 18 (Table 3). All individuals showed water-soaked lesions indicating susceptible reaction to Races 7 and 18; however, quantitative disease severity of individual plants ranged from 6 to 10 (Figure 1, g and h). The effect of B_3 on reaction to *Xcm* Races 7 and 18 was strictly additive ($d/a = 0.01$), differing from "partial dominance" of its effect on *Xcm* Races 2 and 4.

Empire B2b6 population: The region near *G1219* detected in the Empire B2 population also explained 92.2% (LOD 53.36) of the phenotypic variation in reaction to *Xcm* Races 2 and 4 in Empire B2b6 (Table 3).

TABLE 3

Biometric parameters of individual QTLs conferring resistance to bacterial blight of cotton

Population	*Xcm* Race[a]	Probable gene identity[b]	Nearest DNA marker	Chromosome or linkage group	LOD	Variation (%)	Mode of gene action[c]			
							a	*d*	*d/a*	
Empire B2	2 (4)	B_2	*G1219*	*20*	103.12	98.0	−3.51	3.56	−1.01	D
Empire B3	2 (4)	B_3	*pGH510a*	*20*	23.21	88.2	−2.70	−1.11	0.41	AR
	18 (7)	B_3	*pGH510a*	*20*	10.56	53.4	−0.75	−0.01	0.01	A
Empire B2b6	2 (4)	B_2	*G1219*	*20*	53.36	92.2	−3.60	3.50	−0.97	D
	18 (7)	Qb_{6a}	*A1666*	U01	3.32	26.9	−2.74	−0.85	0.31	RA
	18 (7)	(Qb_{6b})	*P12-20a*	*5*	3.07	22.4	0.52	−3.46	−6.65	D
	18 (7)	Qb_{6c}	*pAR827*	D04	3.53	19.4	−1.78	−2.40	1.35	R
	18 (7)	(Qb_{6d})	*pAR723*	*14*	3.01	16.3	1.53	−1.99	−1.30	D
S295	18 (2, 4, 7)	B_{12}	*pAR043*	*14*	50.46	94.2	−3.66	3.49	−0.95	D

A, additive; D, dominant; R, recessive (Paterson *et al.* 1991).

[a] Parentheses indicate *Xcm* race phenotypes that were highly correlated with the *Xcm* race phenotypes used to calculate biometric parameters.

[b] Parentheses indicate that the QTL had an effect opposite to that expected from the parental phenotype.

[c] Biometric parameters were calculated using "recessiveness" and "dominance" to refer to the behavior of the *G. hirsutum* alleles. *a*, (HH − BB)/2; *d*, H/B − [(HH + BB)/2)]. Mode indicates the gene action of individual QTLs with the *G. hirsutum* allele. When two modes of gene action could not be deemed unlikely, the more likely mode was listed first.

The GH allele increased resistance and showed dominant gene action, as observed in the Empire B2 population. Scored as a discrete genetic locus, this trait mapped to an interval between *A1701b* and *pAR377* (Figure 2), again reinforcing the need for quantitative phenotypes to obtain reliable map positions. Forcing the discrete genetic locus into the QTL likelihood interval inflated the distance between *pAR335b* and *G1219* by 7.4 cM (from 31.3 to 38.7 cM).

The genetic basis of resistance to *Xcm* Races 7 and 18 was unexpectedly complex. Four QTLs (Qb_{6a}, Qb_{6b}, Qb_{6c} and Qb_{6d}) collectively explained 56.4% of the phenotypic variation in reaction to *Xcm* Races 7 and 18 (Figure 2; Table 3). Reduced models had LOD reductions of 2.0 or more, so the actions of these genes were considered largely independent. The unexpectedly high complexity of b_6 resistance presumably accounts for the deviation from simple segregation models (Table 2). An initial scan of the genome detected two QTLs. A region (Qb_{6a}) near the DNA marker *A1666* on a linkage group of unknown subgenomic origin (U01) (Figure 2) explained 23.5% (LOD 3.32) of the phenotypic variation in reaction to *Xcm* Races 7 and 18 (Table 3). The GH allele increased resistance in a manner that was partially recessive (dominant gene action could be ruled out, but additivity could not). The region (Qb_{6b}) near marker *pAR1-28* on the A-subgenome chromosome 5 (Figure 2), mapped in a region that is homoeologous to the B_2 locus (on chromosome *20*) and explained 22.4% (LOD 3.07) of the phenotypic variation in reaction to *Xcm* Races 7 and 18 (Table 3). This region may correspond to the bacterial blight resistance gene B_2, discovered in "A" genome species *Gossypium arboreum* that has previously been assigned to chromosome 5 using cytological stocks (Endrizzi *et al.* 1984). The GH allele decreased resistance in a manner that was dominant. Qb_{6a} and Qb_{6b} together explained 38.6% of phenotypic variation at a LOD score of 5.52.

Fixing the effect of Qb_{6a} uncovered two additional QTLs. A region (Qb_{6c}) near DNA marker *pAR827* on D-subgenome linkage group D04 (Figure 2) explained 19.4% (LOD 3.53) of phenotypic variation in reaction to *Xcm* Races 7 and 18 (Table 3). The recessive GH allele increased plant resistance. The region (Qb_{6d}) near DNA marker *pAR723* on D-subgenome chromosome *14* (Figure 2) explained 16.3% (LOD 3.01) of variation in reaction to *Xcm* Races 7 and 18 (Table 3). The dominant GH allele decreased plant resistance.

Although prior studies have suggested possible nonlinear interactions between *Xcm* resistance genes (Knight 1953), we found no such interactions among the QTLs associated with b_6 resistance.

Based on qualitative classification, resistance to *Xcm* Races 7 and 18 in the Empire B2b6 population mapped approximately 30 cM from Qb_{6a}, at the end of linkage group U01 (Figure 2). Because the location (Qb_{6a}) inferred from QTL interval analysis mapped to the end

of the chromosome, it was not possible to assess the "inflation" in size of an interval as a result of adding b_6 as a discrete phenotype.

Chi-squared contingency analysis of segregation data for the RFLP markers closest to Qb_{6b} and Qb_{6d} (*pAR723* and *pAR1-28*) showed highly significant ($P = 8.55 \times 10^{-5}$) deviation from the Mendelian expectation. This interaction appeared to be because of differential survival of particular genotypes—among the GB homozygotes at *pAR723,* only 22% were heterozygous for *pAR1-28,* although *pAR1-28* heterozyotes comprised 53% of the overall population. No linkage disequilibrium (pseudolinkage) was found between the loci, as reflected by a D statistic (Lewontin and Kojima 1960) of −0.00003, not significantly different from zero.

S295 population: A region near the DNA marker *pAR043* on D-subgenome chromosome 14 accounted for 94.2% (LOD 50.46) of phenotypic variation in reaction to each of the four *Xcm* races (Figure 2; Table 3). The locus corresponded closely to that of Qb_{6d} in the Empire B2b6 population. The GH allele was dominant. When scored as a discrete genetic locus, the trait mapped 11.4 cM outside the likelihood interval (suggesting as few as 6% escapes, since each escape would be interpreted as a "double recombinant" in genetic linkage analysis). Forcing the discrete genetic locus into the interval containing the likelihood peak inflated the distance between *pAR043* and *pAR129* by 28.3 cM (from 19.2 to 47.4 cM).

Subgenomic distribution of major resistance genes and QTLs: Bacterial blight resistance genes have been utilized from several sources. B_2 and B_3, initially described and utilized by Knight, were discovered in tetraploid cotton (Knight 1944, 1948a), as was B_{12} (Girardot *et al.* 1986; Wallace and El-Zik 1989, 1990). The b_6 gene was introgressed into *G. hirsutum* from the A-genome diploid species *G. arboreum* (Knight 1953). However, we found three additional genes segregating in the b_6 population we studied. For two of these (Qb_{6b} and Qb_{6d}), the favorable allele curiously derives from the susceptible parent (*G. barbadense*), which is not known to contain introgressed genes from any diploid taxon. Finally, Qb_{6c}, on a D-subgenome linkage group (D04), is highly unlikely to be derived from *G. arboreum.* Like the B_2, B_3, and B_{12} genes, the three QTLs are likely to have evolved at the tetraploid level, perhaps in the course of improving cultivated cotton.

Linkage mapping shows that five (71%) of the seven bacterial blight resistance alleles mapped in this study, including all three (100%) discrete alleles and two (66%) of the three QTLs that originated from tetraploid cotton, mapped to D-subgenome chromosomes. The B_2, B_3, and B_{12} phenotypes were each accounted for by major genes that mapped to D-subgenome chromosomes. For the b_6 phenotype, it seems likely that Qb_{6a}, on a linkage group of uncertain genomic affinity, is the classical b_6 locus (see discussion). In addition, three QTLs have been found in tetraploid cotton, two in the D-subgenome, and one in the A-subgenome.

DISCUSSION

Polyploid formation appears to have conferred new avenues of response to selection for disease resistance in cotton. Among the six alleles (discrete plus QTLs) for *Xcm* resistance loci that appear to have arisen in tetraploid cottons, five (83%) mapped to D-subgenome chromosomes. A simple binomial calculation of the probability of this degree of bias being observed if the two subgenomes are equally likely to spawn R alleles—"one or fewer successes in six trials, where $P = q = 0.5$"—yields a likelihood of only 0.0156, suggesting that the D-subgenome of tetraploid cotton has a higher propensity to give rise to new R-gene alleles.

The complexity of the cotton/*Xcm* relationship is reflected in the discovery of both "horizontal" and "vertical" resistance components, especially regarding the B_3 and b_6 gene systems. One QTL (Qb_{6d}) accounting for only 16.3% of variation in *Xcm* reaction in one population, corresponds in location to a discrete locus accounting for 94.2% of *Xcm* reaction in a different population (B_{12}). Isolation of the discrete allele at the B_{12} locus might also yield the Qb_{6d} QTL, in a manner that has previously been suggested (Robertson 1985).

Incongruence between qualitative and quantitative approaches to mapping: Lack of correspondence in location of bacterial blight resistance genes/QTLs mapped by qualitative and quantitative methods is likely to be explained by a modest number of false-positive or false-negative results ("escapes," or unintended plant-to-plant disease spread, respectively). In linkage analysis, each misscored qualitative phenotype would be interpreted as two recombination events flanking the locus. The "maximum-likelihood" location of a discrete phenotype that included a low frequency of errors would be either in an interval that was large enough to include several "double recombinants," or at a sufficient "recombinational distance" from the end of a linkage group that escapes might be attributed to recombination. The tendency of qualitative scores for bacterial blight resistance genes to map to large gaps in linkage groups, or at the termini of linkage groups is consistent with this expectation. Further, the map inflation (ranging from 4.3 to 28.3 cM) that resulted from forcing discrete resistance scores into the map intervals containing their likelihood peaks is also consistent with varying degrees of misclassification into genotypic classes, based on observation of phenotypes. Even for high-heritability traits such as these, analysis of a quantitative phenotype can improve the reliability of genetic mapping data.

The value of analyzing quantitative phenotypes is especially well-illustrated by the molecular dissection of resistance to *Xcm* Races 7 and 18 in the Empire B2b6 population. Classic data suggested that a single genetic

locus, named b_6, conferred this resistance. Indeed, if mapped as a discrete trait, we could find evidence of only a single locus. However, analysis of more comprehensive quantitative data revealed three additional loci associated with this phenotype.

The quantitative phenotypes we have mapped are of sufficient accuracy to study evolutionary patterns (such as subgenomic distribution) or to establish diagnostic DNA markers for plant breeding programs. However, for applications such as positional cloning that require high precision, progeny testing is strongly recommended to verify the *Xcm* genotype of key "recombinants" thought to be near the gene.

A recessive resistance allele departs from the patterns of most plant resistance genes: In most cases, genetic mapping corroborated classic predictions regarding the number of genes and gene action conferring resistance to various *Xcm* races. Only the "b_6 phenotype" conferring resistance to *Xcm* Races 7 and 18 exhibited numerous inconsistencies, as noted above. The complexity of the b_6 phenotype, involving four genes with very different dosage effects and derived from different parents, presumably explains the deviation from a simple segregation model that we observed. Based on classic evidence that the true b_6 allele is recessive and derived from an A-genome diploid (hence not likely to be on a D-genome chromosome), it most likely corresponds to Qb_{6a}, on linkage group U01 of uncertain subgenomic origin. Since b_6 was derived by introgression from *G. arboreum* (Knight and Clouston 1939; Knight 1946, 1955), this suggests that LG U01 is an A-subgenome group, an interpretation that is neither supported nor contradicted by alloallelic data (Reinisch *et al.* 1994). Loss of function by the recessive b_6 allele (putatively Qb_{6a}) appeared to uncover allelic variation at three additional loci (Qb_{6b}, Qb_{6c} and Qb_{6d}). The validity of these QTLs is supported not only by their LOD scores (>3.0), but also by their correspondence in location to major resistance genes in other populations. Qb_{6d} corresponds closely to the B_{12} locus on chromosome *14* in the S295 population. Qb_{6b} mapped to a region of chromosome *5* that is homoeologous to the B_2 resistance gene on chromosome *20* (Figure 2) and may correspond to B_4, a resistance gene which has previously been assigned to chromosome *5* by classic techniques (Endrizzi *et al.* 1984).

Unexpectedly, enhanced resistance was conferred by two alleles from the susceptible parent (GB), specifically Qb_{6b} on the A-subgenome and Qb_{6d} on the D-subgenome. This parallels recent discoveries of valuable QTLs from unexpected places, such as genes for increased yield from low-yielding wild relatives (Xiao *et al.* 1996). This is an outcome that could not be predicted based on parental phenotypes and further demonstrates the complexity of b_6 resistance. The two GB alleles would not have played a role in the introgression of b_6 from *G. arboreum* into GH (Knight 1953), since GB did not enter into this pedigree.

Knight (1953) reported that the b_6 locus modified the expression of the B_2 locus by increasing plant resistance and reducing susceptibility to *Xcm*. However, our molecular analyses of reaction to *Xcm* Races 2 or 4 in the Empire B2b6 population do not support these findings. Segregation ratios in this population did not fit a two locus (dominant/recessive) model (Table 2). The QTL likelihood map supports a single genetic locus (B_2) explaining 92.2% of the phenotypic variation to *Xcm* Races 2 and 4. If one of the b_6 QTLs conferred resistance to *Xcm* Races 2 or 4, its effects may have been obscured by the discrete effects of the B_2 gene.

"Horizontal components" of bacterial blight resistance: The complexity of b_6 resistance was an unexpected discovery that demonstrates a horizontal component of bacterial blight resistance. Four regions of the genome affected reaction to *Xcm* Races 7 and 18. A combination of QTLs produces a level of resistance equivalent to that conferred by discrete "major" gene resistance. The variation explained by individual b_6 QTLs averaged 21.3%, while the discrete loci imparting resistance to other *Xcm* races accounted for 93.2% on average.

Both the B_2 and B_3 alleles conferred discrete resistance to early *Xcm* Races 2 and 4, but the B_3 allele alone also reduced the severity of damage caused by virulent *Xcm* Races 7 and 18, which were thought to have "defeated" the B_3 resistance mechanism. Flor's "gene-for-gene" hypothesis states that a resistance gene in the plant has a corresponding avirulence gene in the pathogen (Flor 1946, 1947). In some cases, plant resistance proteins interact directly with avirulence proteins from the pathogen (Tang *et al.* 1996; Scofield *et al.* 1996), leading either to "recognition" and a hypersensitive response, or to "lack of recognition" and a susceptible reaction. The finding that B_3 explains variation in severity of damage caused by virulent races of the pathogen may indicate that B_3 functions after recognition, or that *Xcm* Races 7 and 18 can overcome the active defense of B_3. Although B_3 does elicit a hypersensitive response in *Xcm* Races 2 and 4, indicative of a recognition protein (Gabriel *et al.* 1986), the gene product appears to have both qualitative and quantitative activities against different *Xcm* races. An alternative explanation of the effects of B_3 on *Xcm* Races 7 and 18 that cannot be ruled out based on our present data is that the inoculation method used (all four pathogenic races inoculated on the same leaf) resulted in the induction of some sort of local acquired resistance—however, any such resistance was specifically conferred by the B_3 allele, since the attenuation was detected in a segregating population. Further, the B_2 allele for resistance to the same *Xcm* races (2 and 4) showed no such effect on races 7 or 18, contraindicating this alternative.

It is important to note that apparent correspondence of different resistance alleles to common genomic regions, or "homoeologous" regions duplicated as a result

of polyploidy, may reflect the existence of clusters of related genes associated with *Xcm* reaction as has been reported for several other diseases (Martin *et al.* 1994; Zhou *et al.* 1995). Such a cluster would be one possible explanation for different "gene dosage" effects of the B_3 genomic region on *Xcm* Races 2 (or 4) and 18 (or 7). The effect of B_3 on reaction to *Xcm* Races 7 and 18 was strictly additive, somewhat different from "partial dominance" of its effect on Races 2 and 4. This discrepancy may suggest the presence of two closely-linked loci conferring resistance to *Xcm* Races 2 (or 4) and 18 (or 7), respectively. However, this model would require that the source of B_3 contained favorable alleles at both loci.

Subgenomic distribution of mapped genes/QTLs and intergenomic interactions suggest that polyploid formation may have created novel avenues of response to selection: Among the six resistance genes derived from tetraploid cottons, five (83%) mapped to D-subgenome chromosomes—if each subgenome were equally likely to evolve new R-gene alleles, this level of bias would occur in only about 1.6% of cases. The D-subgenome bias suggests that polyploid formation has offered novel avenues for evolution of R-gene alleles.

Possible reasons why the D-subgenome of tetraploid cotton might evolve new R-alleles more rapidly than the A-subgenome include biogeographic considerations, differences in evolutionary rates between subgenomes, gene conversion or other intergenomic exchanges that escaped detection by genetic mapping, or other factors. Biogeographic considerations stem from the possibility that the Old World A-genome diploids may have coevolved with the pathogen. The hypothesized African origin of *Xcm* suggests that A-genome cottons may have had much longer exposure to the pathogen than the New World D-genome cottons, and therefore already contained alleles that conferred resistance when polyploid formation occurred. New mutations in the D-subgenome of tetraploid cotton, putatively less subject to selection for *Xcm* resistance in the wild, may have more frequently created new R-gene alleles that conferred host plant resistance to new pathogenic races. This model implies that D-subgenome QTLs may correspond to homoeologous A-subgenome sites that already contain favorable alleles—an implication that is supported by the finding that the only QTL (Qb_{6b}) we mapped that originated in an A-genome diploid cotton, was indeed homoeologous to a D-subgenome QTL (B_2) that originated in tetraploid cotton.

The biogeographic model, suggesting that a larger number of favorable alleles are fixed in the A-genome as a result of prior natural selection, is weakened by the observation that many D-genome diploid taxa show some degree of resistance to *Xcm*. In a fairly comprehensive survey of the Gossypium genus, "complete immunity" (no visual sign of disease) to *Xcm* Races 1 and 2 was found only in the two Old World species, *G. arboreum* and *G. herbaceum*. Knight also observed varying degrees of *Xcm* resistance in six of the seven D-genome taxa he studied.

Several other factors may also contribute to the D-subgenome bias of new R-gene alleles. In principle, a higher underlying mutation rate in the D-subgenome could account for this observation—a *ca.* 10% higher level of DNA polymorphism in the D-subgenome has been suggested based on RFLP data (Reinisch *et al.* 1994), but is unlikely to account for a bias of the magnitude observed. Non-homologous chromosomal rearrangements (such as A-genome chromatin being transferred to D-genome chromosomes) do not appear to have been a major factor influencing the organization of the modern tetraploid cotton genome (Reinisch *et al.* 1994), but cannot yet be ruled out, especially in genomic regions small enough to escape detection by DNA markers. The possibility of a higher gene number in the D-subgenome is unlikely—the number of RFLP loci, and recombinational length for each subgenome are approximately equal (Reinisch *et al.* 1994). Individual DNA probes tend to detect a slightly larger number of genomic restriction fragments in the A-subgenome than in the D-subgenome (Reinisch *et al.* 1994).

The joining of two genomes with divergent evolutionary histories into a common nucleus appears to have had important consequences for interactions between the cotton plant and the *Xcm* pathogen. Molecular mapping has revealed that the genetic basis of this host-pathogen interaction is more complex than classic data had suggested, and also that the A- and D-subgenomes have made very different contributions to the coevolution of *Xcm* and cotton. Investigation of the subgenomic distribution of genes in polyploids, which control traits for which the respective subgenome donors differ, may be an important aspect of future investigations in evolutionary genetics.

This work was funded in part by a grant from the Texas Agricultural Experiment Station to K.M.E. and A.H.P., and by grants from the Texas Higher Education Coordinating Board and U.S. Department of Agriculture Plant Genome Program to A.H.P. We thank the associate editor and anonymous reviewers for constructive criticism and well-formulated suggestions.

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Communicating editor: J. A. Birchler