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QTL analysis of leaf morphology in tetraploid *Gossypium* (cotton)

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Abstract Molecular markers were used to map and characterize quantitative trait loci (QTLs) determining cotton leaf morphology and other traits, in 180 F_2 plants from an interspecific cross between a *Gossypium hirsutum* genotype carrying four morphological mutants, and a wild-type *Gossypium barbadense*. The prominent effects of a single region of chromosome 15, presumably the classical "Okra-leaf" locus, were modified by QTLs on several other chromosomes affecting leaf size and shape. For most traits, each parent contained some alleles with positive effects and others with negative effects, suggesting a large potential for adapting leaf size and shape to the needs of particular production regimes. Twenty one QTLs/loci were found for the morphological traits at LOD≥3.0 and P≤0.001, among which 14 (63.6%) mapped to D-subgenome chromosomes. Forty one more possible QTLs/loci were suggested with 2.0≤LOD<3.0 and 0.001<P≤0.01. Among all of the 62 possible QTLs (found at LOD≥2.0 and P≤0.01) for the 14 morphological traits in this study, 38 (61.3%) mapped to D-subgenome chromosomes. This reinforces the findings of several other studies in suggesting that the D-subgenome of tetraploid cotton has been subject to a relatively greater rate of evolution than the A-subgenome, subsequent to polyploid formation.

Key words DNA markers · Quantitative trait loci (QTLs) · Morphological traits · Okra leaf · Polyploid formation

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Introduction

Leaf morphology can significantly affect yield, quality, maturity, pest preference, canopy penetration of plant growth regulators, and other important production characteristics of many crops, including cotton. Cultivated cotton is dominated by two tetraploid species, *Gossypium hirsutum* and *Gossypium barbadense*, thought to have formed about 1–2 million years ago by hybridization between a maternal Old World "A" genome taxon resembling *Gossypium herbaceum* (2n=2*x*=26) and paternal New World "D" genome taxon resembling *Gossypium raimondii* (Beasley 1940; Wendel 1989) or *Gossypium gossypioides* (Fryxell 1979) (both 2n=2*x*=26).

Tetraploid cottons have two major leaf types: normal and "Okra leaf". Normal leaf is also called broad leaf, and is predominant among cultivated cottons. Okra leaf is also called narrow leaf, and usually has a deeply-cut leaf edge. Okra leaf has been associated with production advantages such as early maturity (Andries et al. 1969; Heitholt 1993), reduced boll rot (Andries et al. 1969; Jones 1982), reduced leaf area index and higher canopy CO_2-uptake per unit leaf area (Kerby et al. 1980), higher light-saturated, single-leaf photosynthesis per unit leaf area (Pettigrew et al. 1993), a shorter sympodial plastochron (Kerby and Buxton 1978), increased numbers of flowers per season (Wells and Meredith 1986), better pesticide penetration (James and Jones 1985), and moderate levels of pink bollworm resistance (Wilson 1990). Because of these advantages, some researchers have placed priority on breeding for high-yielding Okra-leaf genotypes. In some areas of Australia, Okra-leaf cotton now represents 50% of cotton acreage (Thomson 1995;

Heitholt and Meredith 1998). However, not all data supports the use of Okra-leaf cottons. Meredith (1985) compared the yield and yield components of Okra- and normal-leaf lines in six genetic backgrounds and found that leaf type did not consistently affect yield in Mississippi. Wilson (1986) compared Okra- and normal-leaf isolines from seven genetic backgrounds, finding that Okra-leaf types yielded less than their normal-leaf counterparts in Arizona. The yield deficiency of Okra-leaf cotton in the US may be due to lower emphasis on breeding for Okra-leaf types in this country (Heitholt and Meredith 1998).

The objectives of the present study were to use an established RFLP map (Reinisch et al. 1994) to map and characterize QTLs for leaf morphology and other traits, to identify the subgenomic distribution of genetic loci responsible for morphological variation in polyploid cotton, and to provide DNA molecular markers for use in cotton improvement.

Materials and methods

Mapping population

A total of 180 F2-selfed progeny from the F1 of a cross of a *G. hirsutum* genotype homozygous for mutations conferring glabrousness (T_2 ^{arm}), frego bract (fg), okra leaf (L_2^0), and nectariless (ne_1, ne_2) in the background genotype Deltapine 61 (hereafter GH) ×wild-type *G. barbadense* "Sea Island Seaberry" (GB) were studied. GH and GB were homozygous at all marker loci examined. GH was provided by Dr. David Altman, and its development is described elsewhere (Thomson et al. 1987).

Phenotypes

Individual F2 plants were scored for 14 measures of leaf size and shape (Fig. 1), including lobe numbers (lbno), sublobe number on the main lobe (sl1), main-lobe length (L1) and width (W1), second-lobe length (L2) and width (W2), third-lobe length (L3) and width (W3). Second-lobe angle (A2) was measured as the angle between the main lobe and the second lobe, and the third-lobe angle (A3) indicates the angles formed by the second and third lobes. For all measurements of second and third lobes, the averages of the corresponding lobes on opposite sides of the leaf were calculated. For all measurements, averages of two leaves per plant were used. The average number of leaf hairs in a 6 mm (28.26 mm^2)-diameter ring (hair) was determined based on the average of two counts per leaf. The presence or absence of frego bracts subtending the flower (fg), and nectaries on the lower leaf surface (ne1) were each scored as binomial variables. For plants with nectaries, the size of nectaries (ne_2) was also assessed visually (1=small, 2=medium, 3=large) by inspection of several leaves per plant.

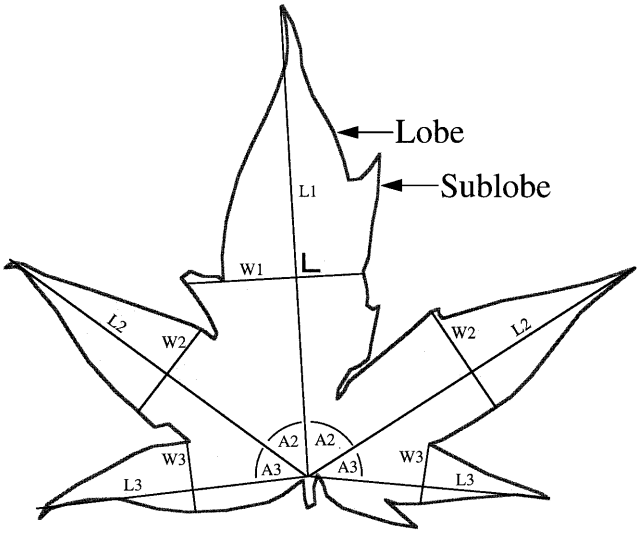

Fig. 1 Diagram of cotton leaf, showing the morphological traits measured

Genotyping and data analysis

RFLP analysis used the laboratory methods and DNA probes previously described (Reinisch et al. 1994), supplemented by new probes (A.H.P. et al., unpublished). Phenotypic distributions for most traits were approximately normal (Fig. 2). Leaf hair number (hair) and main sublobe number (sl1) were skewed left (toward low values), however square-root transformation improved normality, and was used in all analyses. Fg, ne_1, ne_2 and lbno are discrete traits, and were analyzed by a χ^2 contingency test. Trait means and correlations were calculated using SAS (SAS institute, Cary, N.C.) and QGene (Nelson 1997). Linkage maps were made using (MAPMAKER (Lander et al. 1987), and the Kosambi mapping function (Kosambi 1944) was employed to convert recombination frequencies to centiMorgans. Two analytical approaches were used to identify putative QTLs and estimate their phenotypic effects: single-point analysis and interval mapping (Lander and Botstein 1989). For the first procedure one-way ANOVAs were performed by using SAS (SAS Institute 1988), in which marker-genotype groups were used as class variables. For single-point analysis, the program QGene (Nelson 1997) was also used which generates the same results as the routine in SAS but in a convenient tabular form. In order to keep the likelihood of claiming even one false-positive QTL below 5% in the large genome of cotton (26 chromosomes, approximately 4000–5000 cM), we chose a probability level of $P<0.001$ for ANOVA or a LOD score of ≥3.0 as the threshold to assert the presence of a QTL (see Table 2A and Fig. 3). Marker-trait associations with significance values of $0.01 \le P < 0.001$ or $2.0 \le LOD < 3.0$ were deemed "possible QTLs" and were also shown (see Table 2B and Fig. 3). QTL-likelihood maps, gene action, and the phenotypic variance explained (PVE) by both individual-QTL and multiple-QTL models were determined by interval mapping using MAPMAKER/QTL. For each LOD peak, 1- and 2-LOD support intervals were shown for certain QTLs, and maximum-likelihood locations were shown for possible QTLs (see Fig. 3). In cases where the maximum-likelihood location of a QTL fell more than 10 cM from the nearest marker, the LOD score and phenotypic effects reported (see Table 2) were at the marker locus, in order to avoid any possible bias due to artifactual assignment by MAPMAKER/QTL of unlinked genotypic variance to the centers of large intervals between markers.

Results

Genetic map of the interspecific cross

The GH×GB F_2 linkage map included 261 RFLP markers in 26 linkage groups, and spanned 3664 cM with an average spacing of 14.1 cM between markers (Fig. 3). The linear orders of markers showed only small differences from the previously published map (Reinisch et al. 1994; Jian et al. 1998), usually associated with short distances

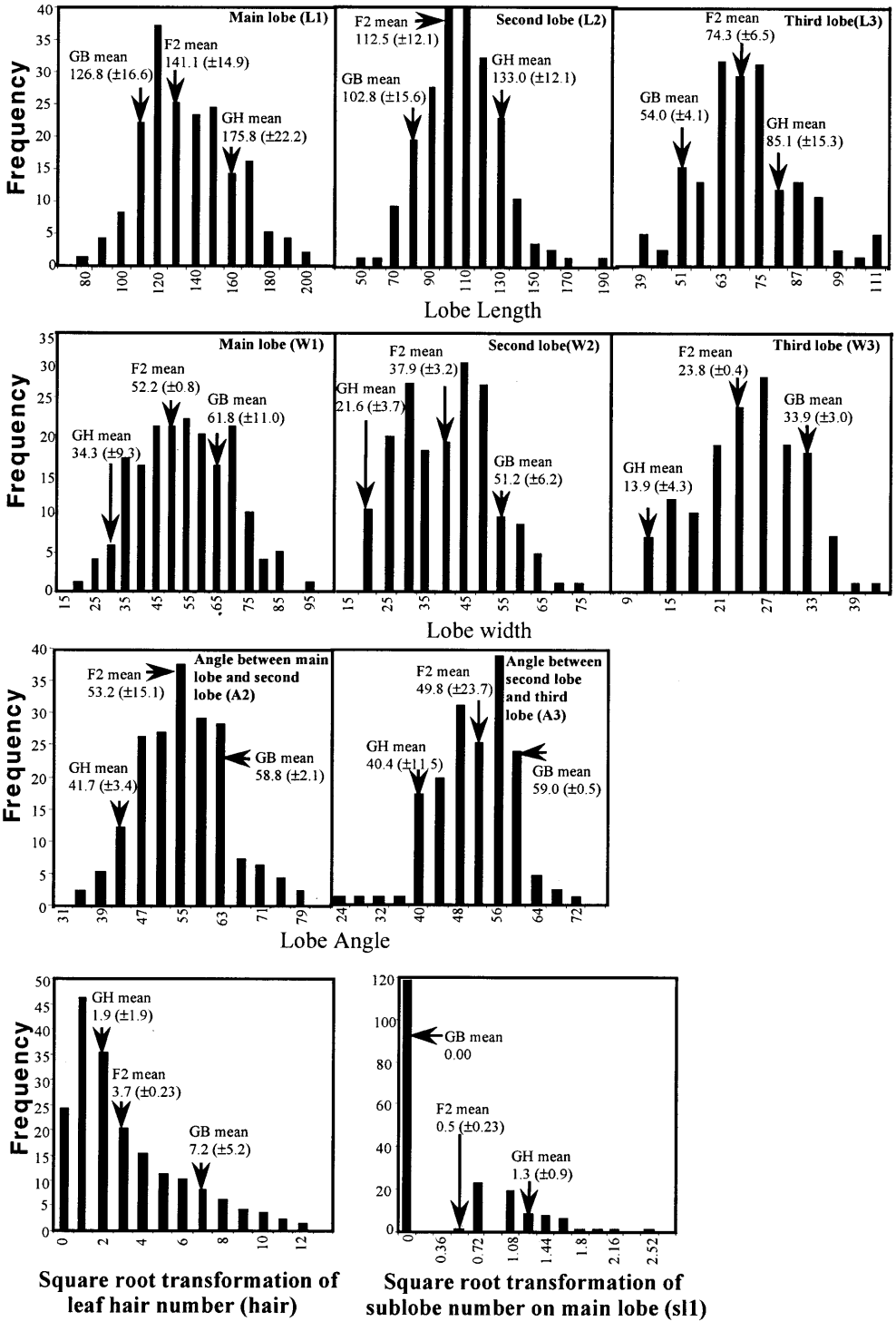

Fig. 2 Frequency distribution for each morphological trait in the F_2 progeny. For trait leaf hair number (hair) and sublobe soluble number (sl1), the results of square root transformation are shown

between markers, or polymorphisms at different homoeologous (duplicated) loci detected by individual probes.

Correlations among traits

Significant ($P<0.01$) correlations were observed between many traits (Table 1). Among the leaf characters, positive correlations were observed between L1, L2 and L3 (r=0.61–0.84) and also between W1, W2 and W3 (r=0.65–0.86). Leaf length and width are also positively correlated, such as L1, L2, and L3 with W1 (r=0.40–0.58); L3 and W2 (r=0.45); and L2 and L3 with W3 (r=0.37–0.65). Other high correlation coefficients were observed for L1 and sl1 (r=0.35), W1 and lbno (r=0.49), W1 and sl1 (r=0.32), L2 and lbno (r=0.46), L2 and sl1 (r=0.32), W2 and lbno (r=0.47), L3 and lbno (r=0.36), L3 and sl1 (r=0.32), W3 and lbno (r=0.37).

Table 1 Correlations between aspects of leaf morphology and other traits in the F_2 population (see Materials and methods for trait abbreviations). Note, r_p=0.01=0.24; r_p=0.05=0.13

Trait	L1	W1	L2	W2	A2	L3	W3	A3	hair	lbno	sl1	fg	ne1
W1	0.40												
L2	0.84	0.58											
W2	0.18	0.86	0.47										
A2	–0.03	0.23	–0.08	0.20									
L3	0.61	0.49	0.71	0.45	–0.11								
W3	0.26	0.65	0.37	0.78	0.10	0.65							
A3	–0.07	0.28	–0.07	0.25	0.25	–0.20	0.21						
hair	–0.12	0.03	–0.15	0.09	0.02	–0.16	0.09	0.13					
lbno	0.17	0.49	0.46	0.47	–0.06	0.36	0.37	0.02	–0.07				
sl1	0.35	0.32	0.33	0.02	0.07	0.32	0.13	0.02	–0.12	0.27			
fg	0.00	0.05	–0.08	–0.09	0.23	–0.17	–0.11	0.15	0.05	–0.09	0.02		
nel	–0.04	–0.15	–0.12	0.19	0.08	0.10	0.03	–0.04	0.09	–0.10	–0.13	0.00	
ne2	–0.07	–0.13	–0.12	0.10	0.04	0.04	0.01	–0.02	0.06	–0.09	–0.11	0.00	0.15

QTLs/genes identified for morphological traits

Marker-trait associations are depicted in Fig. 3. A total of 21 QTLs/loci (Table 2A) affecting morphological traits met the significance threshold (LOD≥3.0, P≤0.001) and an additional 41 possible QTLs/loci (Table 2B) had "suggestive" significance levels of 2.0≤LOD<3.0 and 0.001<P≤0.01.

The proportion of phenotypic variation explained (PVE) by individual marker loci associated with specific QTLs was determined both by regression analysis and interval mapping. In most cases when a QTL mapped close to a specific marker, the R^2 and PVE values were nearly equivalent. When the most-likely position of the QTL (based on interval mapping) resided in the middle of an interval, the PVE estimates from interval mapping were usually higher than those from single-point analysis. In these cases, the PVE values at the nearest marker locus were used.

Lobe length

Three QTLs were detected for the length of the main lobe (L1; Fig. 3; Table 2A), on chromosomes 1 (L1-Chr.1), 15 (L1-Chr.15) and 20 (L1-Chr.20) with LOD ≥3.0 and P≤0.001. Three more possible QTLs were suggested with 2.0≤LOD<3.0 and 0.001<P≤0.01 (Fig. 3; Table 2B), on linkage groups A03 (L1-A03), A06 (L1-A06), and D02 (L1-D02). When the six QTLs were fitted simultaneously using Mapmaker-QTL, they explained 45.3% of the phenotypic variation (Table 2). GB alleles reduce the main-lobe length at three loci (L1-Chr.1, L1-Chr.15, L1-Chr.20) and increase the main-lobe length at the other three loci (Table 2).

The length of the second lobe (L2) was also influenced by the two L1 QTLs on Chromosomes 15 and 20; however, the GB alleles increased length (in contrast to L1), Five more possible QTLs (Fig. 3; Table 2B) were suggested with 2.0≤LOD<3.0 and 0.001<P≤0.01, on chromosomes 5 (L2-Chr.5) and 18 (L2-Chr.18), and linkage groups D01 (L2-D01), D03 (L2-D03) and D04 (L2-D04). The seven L2 QTLs together explained 66.4% of the phenotypic variation (Table 3). The GB alleles reduce the main-lobe length at two loci (L2-D03, L2-Chr.18) and increase the main-lobe length at the other five loci (Table 2).

The length of the third lobe (L3) again reinforced the presence of QTLs on Chrs.15 and 20 – but again showed different allele effects, with the GB allele on Chr.15 increasing length (as for L2) and on Chr.20 decreasing length (as for L1). A possible L2 QTL on linkage group D04 also affected L3, with the GB allele increasing lobe length in both cases. A new QTL was detected on LGD07 (L3-D07), and two more possible QTLs were suggested on chromosomes 10 (L3-Chr.10) and 23 (L3-Chr.23). The six L3 QTLs together explained 64.1% of the phenotypic variation (Table 3). The GB alleles reduce the third-lobe length at four loci (L3-D07, L3-Chr.20, L3-D04, L3-Chr.23) and increase the main-lobe length at the other two loci (Table 2).

Possible homoeology among lobe-length QTLs

The possible L1 QTLs on linkage groups A03 and D02 are in corresponding (homoeologous) regions. The possible L2 QTL on chromosome 5 is in a genomic region homoeologous to the L1/L2/L3-Chr.20 QTL. The possible L2/L3 QTL on linkage group D04 is in a genomic region that is homoeologous to the possible L3-Chr.10 QTL. Although chromosomes 1 and 15 are homoeologous, the two L1 QTLs found on these chromosomes are not in corresponding regions. Based on the coincidence of QTLs affecting related traits (L1, L2 or L3) and possible homoeology, 11 ancestral genomic regions (instead of 14 independent QTLs/genes) derived from the common ancestor of A- and D-genome cottons account for most variation in leaf-lobe length in GH and GB.

Lobe width

Two QTLs were detected for the width of the main lobe (W1; Fig. 3; Table 2A) on chromosome 15 (W1-Chr.15)

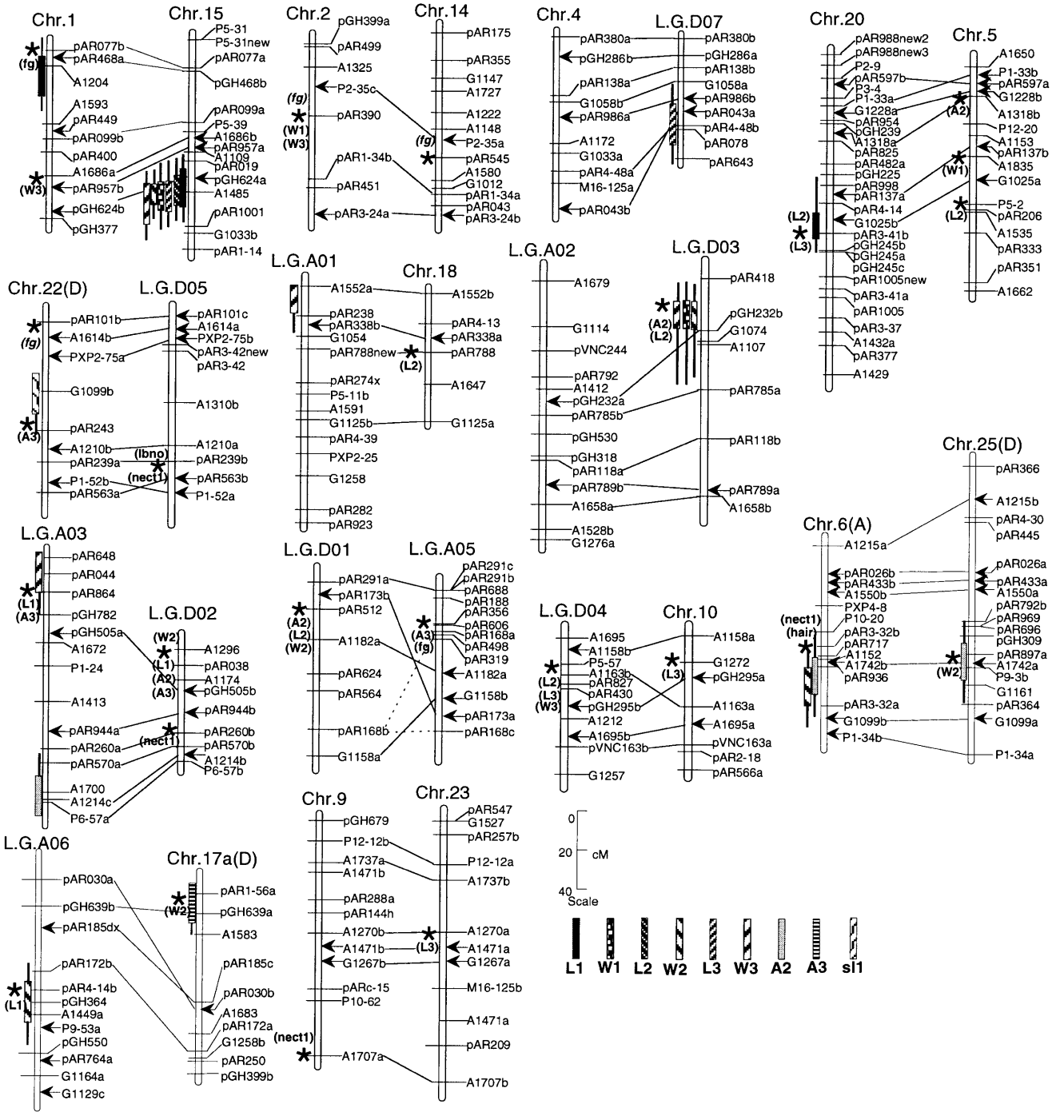

Fig. 3 Marker-trait associations detected. *Bars and whiskers* indicate 1 LOD (10-fold) likelihood intervals for QTLs found with LOD≥3.0. For genes/loci found by the χ^2 test, the gene positions are indicated with *. For the QTLs/loci found at LOD<3.0 and $0.001<P\leq0.01$, the most likely positions are indicated with * and trait name in "()" at the left of chromosome/linkage groups. *Solid lines* connecting probes on different linkage groups indicate homoeologous chromosomal segments supported by three or more pairs of adjacent duplicated loci. *Arrows* indicate the inferred locations of markers used to align the homoeologous linkage groups based on the published map (Reinisch et al. 1994; Jiang et al. 1998)

and LGD03 (W1-D03) with LOD ≥3.0 and $P\leq0.001$. Two more possible QTLs (Fig. 3; Table 2B) were suggested, on chromosomes 2 (W1-Chr.2) and 5 (W1-Chr.5). The four W1 QTLs together explained 59.7% of the phenotypic variation (Table 3). GB alleles reduce the main-lobe width at two loci (W1-D03, W1-Chr.2) and increase the main-lobe width at the other two loci (Table 2).

Two of the W1 QTLs also showed effects on second-lobe width (W2) on chromosome 15 (W2-Chr.15) and linkage group D03 (W2-D03); however, the effects of the W2-D03 QTL are opposite, increasing second-lobe width. Two additional QTLs, on chromosome 6 (W2-

Table 2 Biometrical parameters of individual QTLs affecting cotton morphological traits. A LOD≥3.0 and $P \leq 0.001$; B $2.0 \leq LOD < 3.0$ and $0.01 \leq P < 0.001$. Note: locus name includes trait abbreviation (see Fig. 3) and the linkage group (LG)/chromosome (Chr.). Chromosomes 1–13 belong to the A subgenome, and chromosomes 14–26 belong to the D subgenome. For "linkage groups" that have not yet been assigned to chromosomes, A and D followed by numbers, such as A02 and D03, represent linkage groups previously assigned (Reinisch et al. 1994) to the respective subgenomes. Calculation of additive effects (a,) dominance deviations (d), d/a ratios, and mode of gene action was as described (Paterson et al. 1991). Modes of gene action that could not be deemed unlikely by 1 LOD or more are listed in order of decreasing likelihood. Gene action mode D indicates that the GB allele is dominant

A

Locus	*P*	LOD	PVE	a	d	d/a	Mode
A2-A03	0.00012	3.72	8.80	1.18	0.44	0.37	D
A2-Chr.6	0.00011	3.96	11.80	1.29	0.86	0.67	DA
A2-Chr.25	0.00008	4.10	10.90	1.13	0.01	0.01	D
A3-Chr.17	0.00001	5.04	13.60	0.46	0.37	0.81	A
L1-Chr.1	0.00012	3.92	13.10	– 7.65	12.23	– 1.60	A
L1-Chr.15	0.00013	3.14	11.00	– 9.12	–11.89	– 1.30	R
L1-Chr.20	0.00007	4.25	37.40	–14.04	–39.06	2.78	D
L2-Chr.15	<0.00001	7.85	36.40	9.01	3.86	0.43	A
L3-Chr.15	0.00060	3.22	9.20	0.05	0.10	2.06	A
L3-D07	0.00007	4.15	10.80	– 0.08	0.09	– 1.19	A
s11-Chr.22	0.00063	3.21	8.20	0.40	– 0.17	– 0.42	R
W1-Chr.15	<0.00001	9.25	29.70	9.83	6.55	0.67	D
W1-D03	0.00021	3.55	49.00	– 0.18	20.69	–112.45	R
W2-A06	0.00025	3.60	9.20	– 1.07	0.53	– 0.50	D
W2-Chr.6	<0.00001	9.04	23.20	– 1.61	0.34	– 0.21	DA
W2-Chr.15	<0.00001	20.42	26.80	12.10	3.15	0.26	DA
W2-D03	0.00003	4.53	22.40	– 1.03	17.22	– 16.72	R
W3-A01	0.00020	3.50	27.90	– 0.25	8.88	– 35.52	DA
W3-A03	0.00090	3.05	21.80	– 0.97	– 1.23	1.27	A
W3-Chr.15	<0.00001	11.35	23.00	5.10	4.51	0.88	D
W3-D03	0.00023	3.99	13.50	– 2.26	6.38	– 2.82	R

B

Locus	*P*	LOD	PVE	a	d	d/a	Mode
A2-Chr.5	0.0013	2.91	7.50	– 3.51	0.41	– 0.12	RA
A2-D01	0.0042	2.40	7.30	2.63	– 2.83	– 1.08	RA
A2-D02	0.0023	2.19	8.90	– 2.14	– 3.97	1.86	D
A2-D03	0.0013	2.92	8.60	– 1.88	3.96	– 2.11	R
A3-A03	0.0024	2.62	19.10	– 0.53	– 0.69	1.29	D
A3-A05	0.0024	2.62	10.90	0.05	– 5.02	–102.45	D
A3-Chr.22	0.0042	2.16	9.20	0.92	4.57	4.97	D
A3-D02	0.0035	2.38	8.00	– 2.99	– 0.18	0.06	DA
fg-A05[a]	0.0026	–	–	–	–	–	–
fg-Chr.2[a]	0.0052	–	–	–	–	–	–
fg-Chr.14[a]	0.0091	–	–	–	–	–	–
fg-Chr.22[a]	0.0074	–	–	–	–	–	–
fg-Chr.1[a]	0.00101	–	–	–	–	–	–
hair-Chr.6	0.0015	2.87	34.00	0.66	0.02	0.03	A
L1-A03	0.0015	2.84	19.20	11.82	–12.66	– 1.07	RA
L1-A06	0.0024	2.62	6.80	10.48	5.72	0.55	A
L1-D02	0.0028	2.54	52.40	13.64	–30.62	– 2.24	R
L2-Chr.18	0.0051	2.33	25.90	–10.55	–15.08	1.43	D
L2-Chr.20	0.0048	2.42	7.90	7.61	4.83	0.63	DA
L2-Chr.5	0.0090	2.03	6.70	8.47	– 1.66	– 0.20	A
L2-D01	0.0017	2.77	7.00	3.12	– 0.60	– 0.19	RA
L2-D03	0.0026	2.58	6.60	– 2.08	5.72	– 2.75	D
L2-D04	0.0090	2.20	12.90	6.51	13.59	2.09	R
L3-Chr.10	0.0074	2.15	14.80	6.98	– 5.97	– 0.86	RA
L3-Chr.20	0.0025	2.51	32.20	– 7.62	–17.08	2.24	D
L3-Chr.23	0.0015	2.66	18.80	– 8.26	– 9.40	1.14	D
L3-D04	0.0091	2.04	5.30	– 0.05	– 0.25	5.60	D
lbno-D05[a]	0.0061	–	–	–	–	–	–
nel-D02[a]	0.0072	–	–	–	–	–	–
nel-Chr.6[a]	0.0072	–	–	–	–	–	–
nel-Chr.9[a]	0.0054	–	–	–	–	–	–
nel-D05[a]	0.0053	–	–	–	–	–	–
W1-Chr.2	0.0033	2.54	6.40	– 4.14	– 4.77	1.15	DA
W1-Chr.5	0.0017	2.80	8.40	6.39	2.46	0.38	DA

Table 2 Continued

Locus	*P*	LOD	PVE	a	d	d/a	Mode
W2-Chr.17	0.0028	2.57	6.90	0.01	–1.33	–1.65.75	D
W2-Chr.25	0.0051	2.24	6.30	0.60	–0.77	–1.28	DA
W2-D01	0.0012	3.00	7.40	0.96	–0.62	–0.64	D
W2-D02	0.0077	2.15	5.40	–1.84	–2.81	1.53	DA
W3-Chr.1	0.0091	2.11	12.10	–2.95	1.32	–0.45	RA
W3-Chr.2	0.0085	2.18	8.30	–2.24	–1.58	0.71	DA
W3-D04	0.0039	2.43	8.50	0.04	0.11	3.17	D

[a] Indicates discrete traits analyzed with a χ^2 contingency test; hence, the value of LOD, PVE, a, d, d/a and gene action mode can not be determined

Table 3 Subgenomic distribution of genes controlling morphological traits in tetraploid cotton. Overall PVE of multiple-QTL models is normally slightly less than the sum of the PVEs of the component single-QTL models, due to factors such as nominal confounding of variance explained by different QTLs or interactions between the QTLs

Trait	Total no. of genes[a]	Overall PVE	A subgenome		D subgenome	
			No. genes	PVE	No. genes	PVE
L1	6	45.3	3	31.2	3	25.6
W1	4	59.7	2	14.7	2	47.6
L2	7	66.4	1	6.7	6	61.3
W2	8	73.5	2	22.2	6	58.5
L3	6	64.1	1	14.8	5	54.0
W3	7	74.8	4	63	3	33.1
A2	7	51.3	3	25.1	4	33.9
A3	5	34.6	2	14.5	3	22.6
hair	1	34.0	1	34	0	–
lbno	1	5.7	0	–	1	5.7
sl1	1	11.1	0	–	1	8.2
fg	5	–	3	–	2	–
ne1	4	–	2	–	2	–
Total	62	–	24	–	38	–
%	–	–	38.7	–	61.3	–

[a] Total No. of genes includes those significant at LOD≥3.0/P≤0.001 and 2.0≤LOD<3.0/0.001<P≤0.01

Chr.6) and linkage group A06 (W2-A06) were also found. Four more possible QTLs were suggested with 2.0≤LOD<3.0 and 0.001<P≤0.01 (Fig. 3; Table 2B) on chromosomes 17 (W2-Chr.17) and 25 (W2-Chr.25) and linkage groups D01 (W2-D01) and D02 (W2-D02). When the eight QTLs were fitted simultaneously using Mapmaker-QTL, they explained 73.5% of the phenotypic variation (Table 3). The GB alleles reduce the second-lobe width at four loci (W2-Chr.6, W2-Chr.17, W2-Chr.25, W2-D02) and increase the second-lobe width at the other four loci (Table 2).

Association with third-lobe width (W3) again reinforced the QTLs previously found for W1 and W2 on chromosome 15, and for W1 on linkage group D03. New QTLs were found on linkage groups A01 (W3-A01) and A03 (W3-A03) with LOD ≥3.0 and P≤0.001. Three more possible QTLs were suggested with 2.0≤LOD<3.0 and 0.001<P≤0.01 (Fig. 3; Table 2B) on chromosomes 1 (W3-Chr.1), 2 (W3-Chr.2), and linkage group D04 (W3-D04). When the seven QTLs were fitted simultaneously using Mapmaker-QTL, they explained 74.8% of the phenotypic variation (Table 3). The GB alleles reduce the third-lobe width at five loci (W3-A01, W3-A03, L3-D03, W3-Chr.1 and W3-Chr.2) and increase the third-lobe width at the other two loci (Table 2).

Possible homoelogy among lobe-width QTLs

The W2-Chr.6 QTL and W2-Chr.25 possible QTL are in homoeologous chromosomal regions. The possible W3-Chr.1 QTL and the W1/W2/W3-Chr.15 QTLs are in homoeologous chromosomal regions, as are the W2-D02 QTL and W3-A03 QTL. Although chromosome 17 and linkage group A06 are homoeologous, the QTLs on these linkage groups do not correspond. Based on the coincidence of QTLs affecting leaf width (W1, W2 or W3) and possible homoeology, 12 ancestral genomic regions (instead of 15 independent QTLs/genes), derived from the common ancestor of A- and D-genome cottons, account for most variation in leaf width.

Relationship between lobe length and lobe width

Likelihood intervals coincide closely for W3-A03 and L1-A03, L1-A06 and W2-A06, L1-Chr.1 and W1-Chr.1, L1/L2/L3-Chr.15 and W1/W2/W3-Chr.15, L2-Chr.5 and W1-Chr.5, L2-D01 and W1-D01, L1-D02 and W2-D02, L2-D03 and W1/W2/W3-D03, L2/L3-D04 and W3-D04. Phenotypic effects of some of the loci are compensatory; the GB allele on LGA03 increases lobe length (L1) and reduces lobe width (W3), and the GB alleles on Chr.15 (L2, L3 and W3), LGD02 (L1 and W2), LGD03(L2 and W2) reduce length and increase width. These loci may

not change leaf area but do change leaf shape. The GB alleles at Chr.5 (L2, W1), and linkage groups A06 (L1, W2), D01 (W2, L2) and D04 (L2, L3, W3) are associated with increases in both length and width, increasing leaf area. The GB allele at Chr.1 is associated with reductions in both length (L1) and width (W3), decreasing leaf area.

Leaf-lobe angle

Three QTLs were found for the second-lobe angle (A2) on chromosomes 6 (A2-Chr.6), 25 (A2-Chr.25) and linkage group A03 (A2-A03), with LOD≥3.0 and $P \leq 0.001$ (Fig. 3; Table 2A). Four more possible QTLs were suggested with $2.0 \leq LOD < 3.0$ and $0.001 < P \leq 0.01$ (Fig. 3; Table 2B) on chromosome 5 (A2-Chr.5), and linkage groups D01 (A2-D01), D02 (A2-D02) and D03 (A2-D03). When the seven QTLs were fitted simultaneously using Mapmaker-QTL, they explained 51.3% of the phenotypic variation (Table 3). The GB alleles reduce the second angle at three loci (A2-Chr.5, A2-D02 and A2-D03) and increase the second angle at the other four loci (Table 2).

The genetic control of third-lobe angle (A3) is only partly related to A2, with several different QTLs. Possible A2 and A3 QTLs on linkage group D02 coincided, both reducing lobe angle. One possible A3 QTL on linkage group A03 was associated with the A2-A03 QTL; but the GB allele has opposite effects on A2 and A3. However, a new QTL was detected for A3 (Fig. 3; Table 2A) on chromosome 17) (A3-Chr.17) with LOD≥3.0 and $P \leq 0.001$, and two more possible QTLs were suggested on chromosome 22 (A3-Chr.22) and linkage group A05 (A3-A05). When the five QTLs were fitted simultaneously using Mapmaker-QTL, they explained 34.6% of the phenotypic variation (Table 3). The GB alleles reduce the third angle at two loci (A3-A03 and A3-D02) and increase the third angle at the other three loci (Table 2).

Possible homoeology among lobe-angle QTLs

The A2-A03 together with the A3-A03 possible QTL and the A2/A3-D02 possible QTL are at homoeologous locations. The possible A3-A05 QTL and the possible A2-D01 QTL are in homoeologous chromosomal regions, as are the A2-Chr.25 and A2-Chr.6 QTLs. Based on the coincidence of QTLs affecting lobe angles (A2 and A3) and possible homoeology, seven ancestral genomic regions (instead of ten independent QTLs) derived from the common ancestor of A- and D-genome cottons account for most variation in leaf-lobe angles.

Frego bract (fg)

No loci reached the LOD 3.0 or $P > 0.001$ significance thresholds. Five possible loci were suggested with $0.001 < P \leq 0.01$ (Fig. 3; Table 2B) on chromosomes 1, (fg-Chr.1), 2 (fg-Chr.2), 22 (fg-Chr.22), 14 (fg-Chr.14) and linkage group A05 (fg-A05).

Leaf-hair number (hair)

No loci reached the LOD 3.0 or $P > 0.001$ significance thresholds. One possible QTL was suggested for leaf-hair number (Fig. 3; Table 2B) on chromosome 6 (hair-Chr.6) with LOD=2.87, which explained 34% of the phenotypic variation. The GB allele increased the leaf-hair density (Table 2); this is contrary to the general chracteristics of GB, however it must be remembered that the GH genotype used for this experiment was homozygous for a glabrous mutation.

Lobe number (lbno)

No loci reached the LOD 3.0 or $P > 0.001$ significance threshold. One possible locus was suggested for lbno on linkage group D05 (lbno-D05) with $0.001 < P \leq 0.01$ (Fig. 3; Table 2B).

Main-sublobe number (sl1)

One QTL which explained 11.1% of the phenotypic variation was detected for sl1 on Chromosome 22 (sl1-Chr.22) with LOD ≥3.0 and ≥0.001 (Fig. 3; Table 2A). The GB allele increased the main-sublobe number.

Nectary (ne)

No loci reached the LOD 3.0 or $P > 0.001$ significance thresholds. Four possible loci were suggested for ne_1 (presence vs absence of nectary) on chromosomes 6 (nel-Chr.6), 9 (nel-Chr.9) and linkage groups D02 (nel-D02) and D05 (nel-D05) with $P \leq 0.01$ (Fig. 3; Table 2B). No loci were found for ne_2 (nectary size).

Subgenomic distribution of QTLs/genes

The A-versus D-subgenomic origin of chromosomes and linkage groups was determined based on concordance of tetraploid restriction fragments with those found in only one of the two diploid progenitor genomes. These determinations agreed with prior assignment of cytologically discernible chromosomes to subgenomes in all of the 14 cases for which chromosomal identity of "linkage groups" was known (Reinisch et al. 1994). When the term "linkage group" is used, it means that we do not yet know the identity of the corresponding chromosome (usually due to lack of availability of the diagnostic aneuploid genetic stock).

Among the 62 QTLs/genes affecting cotton morphological traits that met the stringent LOD≥2.0 and $P \leq 0.01$

threshold, 38 or 61.3% (14 or 63.6% at the stringent LOD≥3.0 and $P \leq 0.001$ threshold) fell on D-subgenome linkage groups, which is close to the 71% and 83% of QTLs found on the D-subgenome for other traits, in crosses of the same species (Jiang et al. 1998; Wright et al. 1998).

Discussion

Genetic manipulation of leaf architecture may be a useful breeding tool, with consequences for light interception, canopy CO_2 uptake, and disease and insect preferences cited previously. DNA markers identified herein provide a tool to aid in such manipulations. The discovery that GH and GB each contribute some alleles that increase, and others that decrease, leaf length, width, sublobe angles, and other attributes, suggest that there exists substantial opportunity to breed cottons that transgress the present range of leaf phenotypes found.

Several regions of the genome clearly had effects on more than one trait (Fig. 3; Table 2). Such results may be due to the chance linkage of two, or more, QTLs, or to pleiotropic effects of a single gene on multiple traits. High-resolution linkage maps or the cloning of QTLs are required to definitively distinguish these two possibilities. However, based on the types of traits affected, one can infer whether linkage or pleiotropy is a more likely explanation. QTLs on chromosomes 15 and 20 that influenced both L1 and L2, but with opposite effects – reducing L1 and increasing L2 – may suggest that there may exist "clusters" of functionally related genes at these loci, and that the opposing effects of L1 and L2 may be due to mutations in different members of the cluster.

The region characterized by the largest cluster of QTLs affecting leaf-lobe length and width was found at the lower end of chromosome 15, corresponding approximately to the location of the "Okra-leaf" mutation on the classical map (Endrizzi et al. 1984). These QTLs exerted strong effects on L1, L2, L3, W1, W2, and W3, and somewhat lesser effects on other characters. All of these traits are measures of leaf shape and are strongly positively correlated with one another. It seems likely that allelic variation at the Okra-leaf locus may be affecting all of these traits. The finding that the parental allele effects at this locus vary for different lobes (some positive, some negative) suggest that this may be a complex genetic locus or gene cluster. Previous studies (Endrizzi et al. 1984) indicated that variation in lobe length and width is controlled by alleles at the *l2* locus which is located on the D subgenome. Broad leaf, *l2*, is recessive to the most common Okra-leaf mutant $L2^0$ (Shoemaker 1908), which is found in cultivated cotton and certain wild forms such as *G. hirsutum* race *palmeri* (Stephens 1945). An intermediate form termed sub-okra, $L2^u$, is found in wild *G. hirsutum* and *G. barbadense*. An extreme type, Super-okra, $L2^8$, which develops a single leaf blade at maturity was found as a mutant in Okra leaf and as a variant in the Okra-leaf wild forms of *G. hirsutum* (Stephens 1945). These reported genes conferring lobe length and width may be different alleles at the locus we found here on chromosome 15, or possibly different members of a gene cluster at this locus (if indeed one exists here).

In addition to the Okra-leaf region, the genetic control of leaf length and width was also influenced by several other genomic regions to lesser degrees. In some such cases, multiple phenotypes were affected by the same genomic region; for example, the cluster of QTLs affecting W1, W2 and W3 on linkage group D03. A gene reducing the main-lobe width (W1) may also reduce the width of the second (W2) and third lobe (W3).

The locus "hair-Chr.6" on chromosome 6 is the only gene mapped here affecting trichome density. In a different cotton population, we have previously reported another QTL affecting trichome density that maps to the same region of chromosome 6, exhibiting many of the expected features of the classical t*1* locus (Wright et al. 1999).

Several discrete traits appeared to be segregating in this population, but could not be mapped to single genetic loci. For example, the nectariless trait, thought to be controlled by homoeologous loci on chromosomes 12 and 26 (Endrizzi et al. 1984), could not be mapped with certainty. Four possible loci were suggested on chromosomes 6, 9, and linkage groups D02 (nel-D02) and D05 (nel-D05). None of these could correspond to chromosome 12 (an A-subgenome chromosome). Only two (D02, D05) could correspond to chromosome 26 (a D-subgenome chromosome); however, the limited data available from aneuploid stocks suggest that a different linkage group, D07, may be chromosome 26 (A.H.P. et al., unpublished). Frego bract (fg), previously on chromosome 3 (Endrizzi et al. 1984), also could not be mapped with certainty. Possible marker associations were found on chromosomes 1 (fg-Chr.1) 2 (fg-Chr.2), 22 (fg-Chr.22), 14 (fg-Chr.14) and linkage group A05 (fg-A05). Only A05 could be chromosome 3, for which we do not have an aneuploid stock. Resolution of the locations of the nectariless and frego bract traits will require further study.

Curiously, among the 62 genes mapped, thirty eight (61.3%) mapped to D-subgenome chromosomes. This level of D-subgenome bias is much higher than the expected percentage; if the two subgenomes equally evolve new alleles for these morphological traits, this level of bias would occur in only about 1.6% of cases. These data reinforce other results (Jiang et al. 1998; Wright et al. 1998) which suggest a higher level of allelic variation in the D-subgenome of tetraploid cotton than in the A-subgenome. Possible explanations for the higher level of allelic variation in the D-subgenome than the A-subgenome include multiple origins of the polyploid; differences in evolutionary rates between subgenomes, perhaps associated with different selection pressures prior to polyploid formation; gene conversion or other intergenomic exchanges that escaped detection by genetic mapping; or the spread to the D-subgenome of new mutagenic agents such as dispersed repetitive DNA elements from the A-subgenome (Zhao et al. 1998).

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