TESTS FOR THE ASSOCIATION OF MARKER LOCI WITH CHROMOSOMES IN *GOSSYPIMUM HIRSUTUM* BY THE USE OF ANEUPLOIDS¹

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THE first linkage group in cotton, *Gossypium hirsutum* L., to be located on its specific chromosome was reported by ENDRIZZI (1963). He placed the pilose-brown lint (H_2Lc_2) linkage group on chromosome 6 of the A genome. He also reported negative association tests for eight other dominant marker loci with six primary monosomes and one tertiary monosome. This paper reports further testing of several marker loci with six monosomes and one telocentric. It also reports the identification of a new monosome.

MATERIALS AND METHODS

The aneuploids used in this study were haplo-1, haplo-2, haplo-4, haplo-6, haplo-16, a stock haplo for the long arm of 17, but diplo for the short arm of 17 (this stock is hereinafter referred to as haplo-17_L), and haplo-18. All of the monosomes except haplo-16 have been previously reported (ENDRIZZI and BROWN 1964). Data bearing on the identification of this newly reported monosome are presented in RESULTS AND DISCUSSION. Haplo-17_L was derived through misdivision from haplo-17, and is deficient for the long arm.

The loci tested, and the stocks contributing the critical allele(s), are as follows: B_2 , B_3 , B_4 —bacterial blight resistance genes. These alleles were present in two stocks furnished by DR. LUTHER S. BIRD. Y_1 —yellow petal. Texas Marker Stock (TM)—586, a multiple dominant stock, was used as source for the Y_1 allele. bw —withering bract. cu , fg , cl_1 , v , gl_1 —cup leaf, frego bract, cluster fruiting, virescent yellow, and glandless stem. These recessive alleles are present in TM-582, a multiple recessive stock. chl_1 , chl_2 —the duplicate recessive loci conditioning chlorophyll deficiency. The recessive alleles originally were derived from the *G. hirsutum* × *G. barbadense* cross. L^L —lacinated leaf. Duplicate A-genome locus of L^o in the D genome. ml —mosaic leaf (LEWIS 1958).

The monosomes identified thus far in the series have been placed in Texas Marker Stock 1 (TM-1) background by backcrossing. TM-1 is a highly inbred commercial-type line of *G. hirsutum*, and carries "wild-type" or standard alleles of mutant loci. TM-1 was used as the recessive tester stock for dominant mutant loci; the contributing stocks themselves were used as tester stocks for the recessive mutant loci. Since the monosomic plants are phenotypically distinguishable from their disomic siblings (BROWN and ENDRIZZI 1964), cytological analyses were not made in the studies reported here.

With one exception, the method of study used to determine association or independence of loci and chromosomes was the study of the test cross progenies. After obtaining the critical allele in monosomic background by crossing the standard monosomes with the allele-carrying marker

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stocks, the F_1 monosomes were testcrossed reciprocally with recessive tester stocks. Association was determined by observing coincidence of cytotype and phenotype. This general procedure has been described by CLAUSEN and CAMERON (1944), SEARS (1953) and ENDRIZZI (1963).

The exception mentioned was the method used in the study of the duplicate recessive loci chl_1 , chl_2 . Since chlorophyll deficient plants (of genotype chl_1 chl_1 chl_2 chl_2) with a predominantly *hirsutum* background usually die at an early seedling stage, an F_2 analysis (SEARS 1953) was used.

Inoculation and scoring of plants in the study of the B_2 , B_3 , and B_4 genes was kindly accomplished by DR. LUTHER S. BIRD of this station. The test progenies were inoculated at first anthesis with a mixture of race 2, and variants 4 and 6 of *Xanthomonas malvacearum* (EF.Sm.) Dowson. Disease grading was done about 3 weeks later, using a scale of 1, completely resistant, to 10, completely susceptible (BIRD and HADLEY 1958).

Throughout this paper, the testcross progenies are referred to in the following manner: (1) where the monosomic F_1 (carrying the critical allele) was used as female in the cross with its recessive tester stock, this progeny is referred to simply as the testcross progeny. (2) where the monosomic F_1 is used as male in this testcross, this progeny is referred to as the reciprocal testcross progeny. Twenty-plant populations were sought for each testcross and reciprocal testcross progeny. Since $n-1$ male gametes normally fail to function, reciprocal testcross progeny consisted entirely of disomic plants.

RESULTS AND DISCUSSION

Identification of new monosome: In 1961, a monosomic plant, designated M25, was found in the progeny of Z1707 (open-pollinated). Z1707 had the cytotype 24II 4I, and had R_i (red plant body) in its background. The new monosome, which had been scored in the field as $R_i r_i$, was outcrossed to TM-1 (genotype $r_i r_i$ with respect to this locus). The progeny of this cross, grown in 1962, consisted of ten plants, of which seven were analyzed cytologically. These were as follows: 4 plants—26II, with phenotype $R_i r_i$; 1 plant—26II I, with phenotype $R_i r_i$; and 2 plants—25II I, with phenotype $r_i r_i$.

The linkage of the R_i phenotype with $2n$ (or $2n+1$) and the r_i phenotype with $2n-1$ in the progeny of M25 indicated that this monosome carried the $R_i cl_i Dw$ linkage group. Furthermore, in the first backcross generation (M25 r_i $\times$ TM-1), grown in 1963, the monosomic plants had less anthocyanin stem pigment than did their disomic siblings. Reduced pigment also was apparent in the leaf laminae, veins, and pulvini. In general, the monosomic plants showed a brighter green leaf color than was characteristic of the disomics. On the basis of this evidence, M25 was designated haplo-16, since the D genome chromosome carrying the $R_i cl_i Dw$ linkage group had previously been designated chromosome 16 (ENDRIZZI and BROWN 1964). Subsequent data gathered in connection with another study (ENDRIZZI and KOHEL, unpublished) have since confirmed this identification.

Bacterial blight resistance genes B_2 , B_3 , and B_4 : The results of the test of the B_2 and B_3 genes against six monosomes and haplo-17_L, and the B_4 gene against the four A genome monosomes (B_4 is known to be in the A genome) are shown in Table 1. With no association between cytotype and phenotype, apparently none of the data show linkage, and except for haplo-17_L, it can be said that none of these genes is on any of the chromosomes tested.

The results of the testing of B_2 and B_3 against haplo-17_L require further ex-

TABLE 1

Progeny classes in testcrosses and reciprocals of monosome lines $\times B_2B_3$ and B_4

Cross	Progeny			
	2n		2n-1	
	Resistant	Susceptible	Resistant	Susceptible
B_2B_3				
F ₁ haplo-1 $\times b_2b_3$	17	2	1	0
reciprocal	14	6	.	.
F ₁ haplo-2 $\times b_2b_3$	7	6	2	5
reciprocal	16	4	.	.
F ₁ haplo-4 $\times b_2b_3$	10	2	4	4
reciprocal	11	9	.	.
F ₁ haplo-6 $\times b_2b_3$	8	7	3	2
reciprocal	15	5	.	.
F ₁ haplo-16 $\times b_2b_3$	10	7	0	1
reciprocal	16	4	.	.
F ₁ haplo-17 _L $\times b_2b_3$	6	5	5	4
reciprocal	14	5	.	.
F ₁ haplo-18 $\times b_2b_3$	7	4	6	3
reciprocal	12	8	.	.
B_4				
F ₁ haplo-1 $\times b_4$	8	9	3	0
reciprocal	8	12	.	.
F ₁ haplo-2 $\times b_4$	8	8	1	3
reciprocal	9	11	.	.
F ₁ haplo-4 $\times b_4$	5	6	5	3
reciprocal	10	10	.	.
F ₁ haplo-6 $\times b_4$	8	8	0	3
reciprocal	10	10	.	.

planation. Data were obtained by KNIGHT (1941) that indicated the B_2 and B_3 loci were on the same chromosome but independently inherited. When B_2 and B_3 are present together in homozygous or heterozygous condition they impart a degree of resistance of about 1 on the 10-point scale. If neither allele is present, a score of about 6 results. If one or the other allele is present alone, a degree of resistance somewhere between 1 and 6 (usually 3 or 4) is imparted. The actual scores obtained in the field for the test progenies of haplo-17_L and B_2B_3 are as follows:

Grade	1	2	3	4	5	6	7
Testcross 2n	5	0	1	2	2	1	0
2n-arm	1	2	2	1	2	1	0
Reciprocal 2n	13	0	1	3	0	0	2

If B_2 and B_3 were on opposite arms of chromosome 17, disomic plants in the test progeny should segregate 1 resistant: 1 partly resistant (none fully susceptible). Plants with an unequal bivalent in the test progeny should segregate 1 susceptible: 1 partly resistant (none fully resistant). Disomics in the *reciprocal*

test progeny should segregate 1 resistant: 1 partly resistant (again, none fully susceptible). The scores obtained indicate that B_2 and B_3 are *not* on opposite arms of chromosome 17, although the data are not completely clear-cut. One can see that if just a few plants had received slightly different scores, a case for B_2 and B_3 being on opposite arms of chromosome 17 would be made. Thus, there remains a possibility that B_2 is on one arm of chromosome 17, while B_3 is on the other.

We *can* say, however, that the B_2 and B_3 loci are not *both* on the long arm of chromosome 17, while it is possible that they are both on the short arm of chromosome 17. Studies are under way with haplo-17 to remove doubts of the possible association of B_2 and B_3 with chromosome 17.

Yellow petal (Y_1): Haplo-1 and haplo-2 have previously been tested with all the loci in multiple dominant stock TM-586 except Y_1 (ENDRIZZI, 1963). Table 2 shows the number of plants in each class of the testcross progeny. Both dominant and recessive phenotypes were present in each of the cytotypes $2n$ and $2n-1$. Also, in the reciprocal testcross, there were both dominant and recessive phenotypes among the disomic plants. From these results it is obvious that the Y_1 locus is not on chromosome 1 or 2.

Withering bract (bw): The plants being used as a source for the bw allele died early (a characteristic of the bw phenotype) and reciprocal testcrosses were not made. However, sufficient seeds were available to have three times as many plants in the testcross progeny as usually are grown. These scores are shown in Table 2. Both bw and normal plants appeared among monosomic and disomic plants alike, indicating that the bw locus is not on chromosome 2.

Cup leaf, frego bract, cluster, virescent, and glandless: Results of the testcross progenies for these recessive loci are shown in Table 3. These data show that none of the loci are on the long arm of chromosome 17, or on chromosomes 1, 2, 4, 6, or 18. While certain class-combinations were absent in some progenies (e.g., there were no $2n-1$ Cl_1 for haplo-2), nevertheless the remaining classes established that no linkage existed. In general, dominant and recessive phenotypes were noted in both cytotypes of the testcross, and both dominant and recessive disomics were noted in the reciprocal testcrosses. These are the results expected when no linkage exists between marker and chromosome.

Duplicate chlorophyll-deficiency loci (chl_1 and chl_2): The relationship of the

TABLE 2

Progeny classes in testcrosses and reciprocals of haplo-1 and haplo-2 $\times$ Y_1 and haplo-2 $\times$ Bw

Testcross	Cytotype	Progeny			
		Y_1	y_1	Bw	bw
F_1 haplo-1 $\times$ tester	$2n$	6	6	..	..
	$2n-1$	4	3	..	..
tester $\times$ F_1 haplo-1	$2n$	13	7	..	..
F_1 haplo-2 $\times$ tester	$2n$	8	3	22	18
	$2n-1$	3	5	7	11
tester $\times$ F_1 haplo-2	$2n$	12	8	..	..

TABLE 3

Progeny classes in testcrosses and reciprocals of monosome lines × recessive mutant alleles in Texas Marker Stock No. 582

Cross	Progeny									
	<i>Cu</i>	<i>cu</i>	<i>Fg</i>	<i>fg</i>	<i>Cl₁</i>	<i>cl₁</i>	<i>V</i>	<i>v</i>	<i>Gl₁</i>	<i>gl₁</i>
F ₁ haplo-1 × tester 2n	10	7	10	7	9	8	6	11	10	7
2n-1	1	2	1	2	1	2	3	0	0	3
reciprocal 2n	11	8	10	9	11	8	5	14	12	7
F ₁ haplo-2 × tester 2n	7	9	6	10	8	8	5	11	6	10
2n-1	2	2	3	1	0	4	2	2	3	1
reciprocal 2n	11	9	12	8	12	8	9	11	11	9
F ₁ haplo-4 × tester 2n	11	4	6	9	4	11	10	5	6	9
2n-1	5	0	4	1	4	1	3	2	3	2
reciprocal 2n	10	10	11	9	13	7	7	13	9	11
F ₁ haplo-6 × tester 2n	7	11	9	9	12	6	10	8	7	11
2n-1	7	6	6	7	1	12	5	8	6	7
reciprocal 2n	2	6	4	4	5	3	6	2	3	5
F ₁ haplo-17 _L × tester 2n	7	11	8	10	8	10	9	9	11	7
2n-1	1	1	0	2	1	1	0	2	2	0
reciprocal 2n	7	11	9	9	12	6	7	11	8	10
F ₁ haplo-18 × tester 2n	8	7	8	7	6	9	8	7	10	5
2n-1	2	3	0	5	3	2	4	1	4	1
reciprocal 2n	8	8	6	10	9	7	7	9	10	6

chlorophyll deficiency loci in *G. hirsutum* and *G. barbadense* is as follows: *G. hirsutum*—*chl₁ chl₁ Chl₂ Chl₂* or *Chl₁ Chl₁ Chl₂ Chl₂*; *G. barbadense*—*Chl₁ Chl₁ chl₂ chl₂*.

Many *hirsutum*-*barbadense* crosses segregate 15 green : 1 yellow in the F₂ generation. In predominantly *hirsutum* background, the deficient genotype results in death at an early seedling stage, but if shaded, a plant may be grown to maturity, at which time it then displays a normal phenotype. Such a plant was used as a source for the *chl₁* and *chl₂* alleles for this test. It was crossed as male onto each of the aneuploid lines tested.

Since our TM-1 lines (in which we maintain the monosomes) has genotype *chl₁ chl₁ Chl₂ Chl₂*, the F₁ of the line monosomic for the chromosome with *chl₂* would be chlorophyll-deficient, if it is expressed in hemizygous condition at the *chl₂* locus. Since all the F₁'s in this case were green, this indicated either no linkage or an inexpressive *chl₂* hemizygote. Of course, if *chl₁* had been linked with one of the monosomes, the F₁ would have been green in any event, since the other locus would be heterozygous. Because of the anticipated difficulty of growing testcross progenies of yellow phenotype to such a stage in the field where phenotypic classification of cytotype could be made, an F₂ analysis was resorted to. Selfed seeds were obtained from monosomic and disomic F₁ plants for each

line, and germinated in the greenhouse where seedlings were scored for chlorophyll deficiency.

The expected ratios of disomic to monosomic plants in these monosomic lines, as measured over several years, are: haplo-2—2.5:1, haplo-4—2.8:1, haplo-6—2.65:1, and haplo-17_L—4.0:1.

If *chl*₂ were linked with any of these monosomes (or arm, in the case of chromosome 17), then yellow would be the predominant class in the F₂ progeny from the monosomic F₁ parent in the critical line (if hemizygous is inexpressive). The ratio of yellow : green would be peculiar to the line, and equal to the ratio of disomes : monosomes for that line; e.g., if *chl*₂ were on chromosome 4, the ratio should be about 2.8 yellow : 1 green.

Table 4 shows the ratio of green : yellow plants in the F₂ progenies. In no case was yellow the predominant class. All F₂ progenies, of both 2n and 2n—1 F₁ parents in each line, segregated in a ratio not deviating significantly from 3:1. In addition, no monosomic ratio differed significantly from the disomic ratio within any line. Thus it can be said that the *chl*₂ locus does not lie on the long arm of chromosome 17, or on chromosomes 2, 4, or 6.

On the other hand, the same conclusion can *not* be drawn for the *chl*₁ locus from these data. If *chl*₁ were linked with any of these monosomes (and not expressed as a hemizygote), then the F₂ progeny of the monosomic F₁ parent would segregate 3 *green* : 1 *yellow* among the disomic plants of the critical line, while the monosomic plants would all be green. This would give expected ratios of *green* : *yellow* as follows: haplo-2—4.6:1; haplo-4—4.4:1; haplo-6—4.5:1; and haplo-17_L—4.0:1.

Rather large populations (up to about 750 plants) would be required to detect

TABLE 4

*F*₂ segregation ratios of monosomic and disomic F₁'s, and chi-square analysis thereof, of haplo-2, 4, 6, and haplo-17_L × *chl*₁ *chl*₂

F ₂ derived from:	Observed			Green:yellow ratio	Chi-square	
	Total	Green	Yellow		Deviation from 3:1	Independence*
F ₁ haplo-2 2n	79	59	20	2.95:1	.01	.52
F ₁ haplo-2 2n—1	64	51	13	3.92:1	.75	
F ₁ haplo-4 2n	76	61	15	4.07:1	1.12	.14
F ₁ haplo-4 2n—1	77	60	17	3.53:1	0.35	
F ₁ haplo-6 2n	98	74	24	3.24:1	.01	.15
F ₁ haplo-6 2n—1	98	71	27	2.63:1	.21	
F ₁ haplo-17 _L 2n	71	53	18	2.94:1	.01	.52
F ₁ haplo-17 _L 2n—arm	74	60	14	4.29:1	1.46	

* Between monosome ratio and disome ratio of green:yellow, for each chromosome or arm (corrected).

TABLE 5

Progeny classes in testcrosses and reciprocals of haplo-1, 2, 4 and 6 × L^L (laciniated leaf)

Cross	Progeny							
	Haplo-1		Haplo-2		Haplo-4		Haplo-6	
	L ^L	l	L ^L	l	L ^L	l	L ^L	l
F ₁ × tester (ll) 2n	13	0	3	5	5	6	2	7
2n-1	0	7	6	6	4	5	10	1
reciprocal 2n	20	0	8	11	10	10	10	10

linkage of *chl*₁ with any of these monosomes, if one works with the genotype of our TM-1 line. However, with monosomes of genotype *Chl*₁ *Chl*₁ *chl*₂ *chl*₂, testing would be as simple as in the case just reported here. Monosomes in *barbadense* background would have this genotype, and such monosomes are in stages of development by ENDRIZZI. We decided to wait and use those *barbadense* monosomes in a test rather than grow out the tremendously large populations required for the *hirsutum* monosomes, to test for the presence of *chl*₁ on monosomes.

Haplo-1, 2, 4, and 6 tested against the laciniated leaf locus, L^L: The results of tests for these monosomes and the lacinate locus are shown in Table 5. For three of the monosomes, haplo-2, haplo-4, and haplo-6, the tests were negative; both phenotypes were present in each cytotype of the testcrosses, while both phenotypes were present in the disomics of the reciprocal testcross. For haplo-1, however, only laciniated disomes and non-laciniated monosomes appeared in the testcross progeny; only laciniated disomes appeared in the reciprocal testcross progeny. This indicates that the L^L locus is on chromosome 1 of the A genome.

Chromosome 15 in the D genome carries the L^o (okra leaf) locus (ENDRIZZI and BROWN 1964). Since L^L is considered to be a duplicate locus of L^o, this would suggest that chromosome 1 and 15 are homologous.

Haplo-4 × mosaic leaf (ml): The F₁ progeny of the cross haplo-4 × *ml ml* consisted of 19 plants. Seven showed the mosaic leaf phenotype at an early seedling stage, while the remaining 12 had normal leaves. Slightly later in the season when the plants were scored phenotypically for cytotype, the mosaic plants were found to be monosomic; the normal plants disomic. This result indicates that the *ml* locus is on chromosome 4.

Since this is the first recessive locus to be located on a specific chromosome by direct monosomic test, our experience with hemizygote expression is not sufficient to know whether positive expression will be the general pattern in cotton. The amphiploid nature of cotton might suggest that it would not. However, any recessive alleles that are expressed in the hemizygous state will be useful in eliminating unidentified monosomic lines from our series, by a one-generation test, without resorting to cytological examination of monosome-translocation combinations. The only other recessive locus tied to its chromosome (by indirect association) is cluster (*cl*₁), as described above in the first section of RESULTS and DISCUSSION. Crosses have been made to determine whether *cl*₁ is expressed

as a hemizygote, and tester stocks being synthesized will carry the recessive alleles of the *ml* and *cl₁* loci.

SUMMARY

A new cotton monosome, haplo-16, is reported. This chromosome carries the *R₁cl₁Dw* linkage group. The bacterial blight resistance genes *B₂* and *B₃* (linked, genome unknown) are independent of chromosomes 1, 2, 4, 6, 16, and 18, and of the long arm of chromosome 17. The blight resistance gene *B₄* (A genome) is independent of chromosomes 1, 2, 4, and 6. Yellow petal (*Y₁*) is independent of chromosomes 1 and 2. The withering bract locus, *bw*, is not on chromosome 2. The recessive mutant loci cup (*cu*), frego bract (*fg*), cluster fruiting (*cl₁*), virescent yellow (*v*), and glandless stem (*gl₁*) are not on chromosomes 1, 2, 4, 6, or 18, or on the long arm of chromosome 17. The chlorophyll deficiency locus, *chl₂*, is not on chromosome 2, 4, or 6, or on the long arm of chromosome 17.

The lacinated-leaf locus, *L^l*, is on chromosome 1. The mosaic-leaf locus, *ml*, is on chromosome 4. Marker loci in cotton which have been located as to specific chromosome may be summarized as follows: the *H₂Lc₂* linkage group is on chromosome 6 (ENDRIZZI 1963); the *R₁cl₁Dw* linkage group is on chromosome 16 (ENDRIZZI and BROWN 1964); the *L^ocr Lg* linkage group is on chromosome 15 (ENDRIZZI and BROWN, 1964); *L^l* is on chromosome 1 and *ml* on chromosome 4, as reported herein.

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