Published November, 1965

Linkage Tests in Upland Cotton, *Gossypium hirsutum* L.[1]

R. J. Kohel, C. F. Lewis, and T. R. Richmond[2]

A PROJECT of long standing at the Texas Agricultural Experiment Station has dealt with the genetic analysis of mutant characters in American Upland cotton, *Gossypium hirsutum* L. The work has involved: *(1)* the orderly testing and documentation of the older and widely recognized mutants which had not been thoroughly analyzed and recorded in the literature, *(2)* the production, acquisition, and analysis of new mutants, and *(3)* the determination of the linkage relationships among the simply inherited mutants. Recently, certain cytologic techniques, particularly monosomic analysis, have proved to be a valuable adjunct to coventional linkage analyses.

In this paper we have attempted to summarize and bring up to date the research on linkage relationships in *G. hirsutum* conducted at this station over the past 20 years. The work of other investigators has been incorporated into the final summary and discussion of linkage in Upland cotton.

MATERIALS AND METHODS

The gene symbols and character names of the stocks used in the College Station experiments are given in Table 1. The first 19 characters listed in the table have been thoroughly tested and documented by publications. The 9 characters remaining, though adequately tested and documented, constitute relatively new or little known characters.

The combinations of characters tested at College Station are shown in Figure 1. The figure also shows, by appropriate symbolization, the tests involving the combinations of characters involved in the College Station experiments that were tested and reported by Stephens (20) and not repeated in the experiments in question. The data and findings of Stephens (20) were taken into account in planning and organizing the College Station linkage experiments to avoid needless repetition or duplication.

Although certain tester stocks combining two or more genetic markers had been in use earlier, it became apparent by 1951 that these—and certainly the "single gene" marker stocks then in unorganized use—were inefficient in terms of large-scale linkage testing. Consequently we set out to develop multiple marker lines, combining all of the proved monogenic recessive characters into one stock and all of the proved monogenic dominant characters into another. After a few years of work toward these ends practical limitations became apparent. As recessive characters were added to a single stock the vigor and productivity of the stock declined; thus, adequate production of experimental material—and even the survival of the stock—became a problem. Furthermore, in both the multiple recessive and multiple dominant stocks, certain character expressions, discrete in individual stocks, became obscure and difficult to distinguish when observed or measured along with certain phenotypically or morphologically similar characters in a common stock. Therefore, several multiple marker lines have been developed. In the present report all but a few of the linkage tests involved three tester stocks:

T582—cl_1, *fg*, *cu*, gl_1, and *v*;
T586—R_2, *N*, Lc_1, L^o, R_1, H_2, *P*, and Y_1;
T588—R_2, *N*, *Lg*, L^o, R_1, H_2, *P*, and Y_1.

T582 is a multiple recessive and T586 and T588 are multiple dominant lines. Certain other tester stocks, carrying one or two markers, were in use prior to the development of the multiple markers and others were developed for specific tests which could not be accomplished with the multiple marker stocks.

Conventional backcross and testcross procedures supplied the greater part of the data used in the linkage analyses. F_2 data were employed for analyses involving tests of recessive loci and for supporting information on other tests.

RESULTS

The results of linkage tests conducted at College Station are presented in summary form in Figure 1. The segregation and tests of significance will not be presented for each combination of characters tested because of the voluminous nature of the detailed data. The results of those tests which showed significant linkage will be discussed in the text and their segregations will be presented in detail. Also for simplicity of presentation, linkage tests for multiple alleles are presented for only one member of a series; the other allelic members are named and discussed in the text.

The upper half of Figure 1 contains the results from backcross populations and the lower half of the figure con-

Table 1. Gene symbol and character name of stocks used in linkage experiments.

Symbol	Name and reference*	Symbol	Name and reference
Lc_1	Brown lint (5)	gl_1	glandless stem (14)
N	Naked seed (5)	P	Yellow pollen (5)
R_2	Petal spot (5)	Rd	Dwarf red (13)
cr	crinkle (5)	v	virescent yellow (5)
L^o	Okra Leaf (5)	Y_1	Yellow petal (5)
Lg	Green lint (5)	ia	accessory involucre (6)
cl_1	cluster (5)	ml	mosaic leaf (12)
Dw	Dirty white (16)	ms_2	male-sterile (18)
R_1	Red plant (5)	Rg	Ragged leaf (8)
H_2	Pilose (19)	vf	veins-fused (9)
Lc_2	Brown lint (21)	(HA-1)†	Heritable abnormality (15)
cn	crenate (also round leaf) (20)	(HA-2)	Heritable abnormality (15)
cu	cup leaf (10)	(Li)	Ligon lintless (1)
fg	frego bract (2)	(rug)	rugose (4)

*Numerals in parentheses following the character name are the usual literature citations and refer to the "authority" who established the characters and their gene symbols, on evidence set forth in corresponding publications listed at the end of this paper. † Symbols in parentheses are not formal designations and are used for convenience only in this paper.

[1] Contribution from the Crops Research Division, ARS, USDA, in cooperation with the Texas Agricultural Experiment Station. Received June 28, 1965.

[2] Research Geneticists and Research Agronomist, Crops Research Division, ARS, USDA.

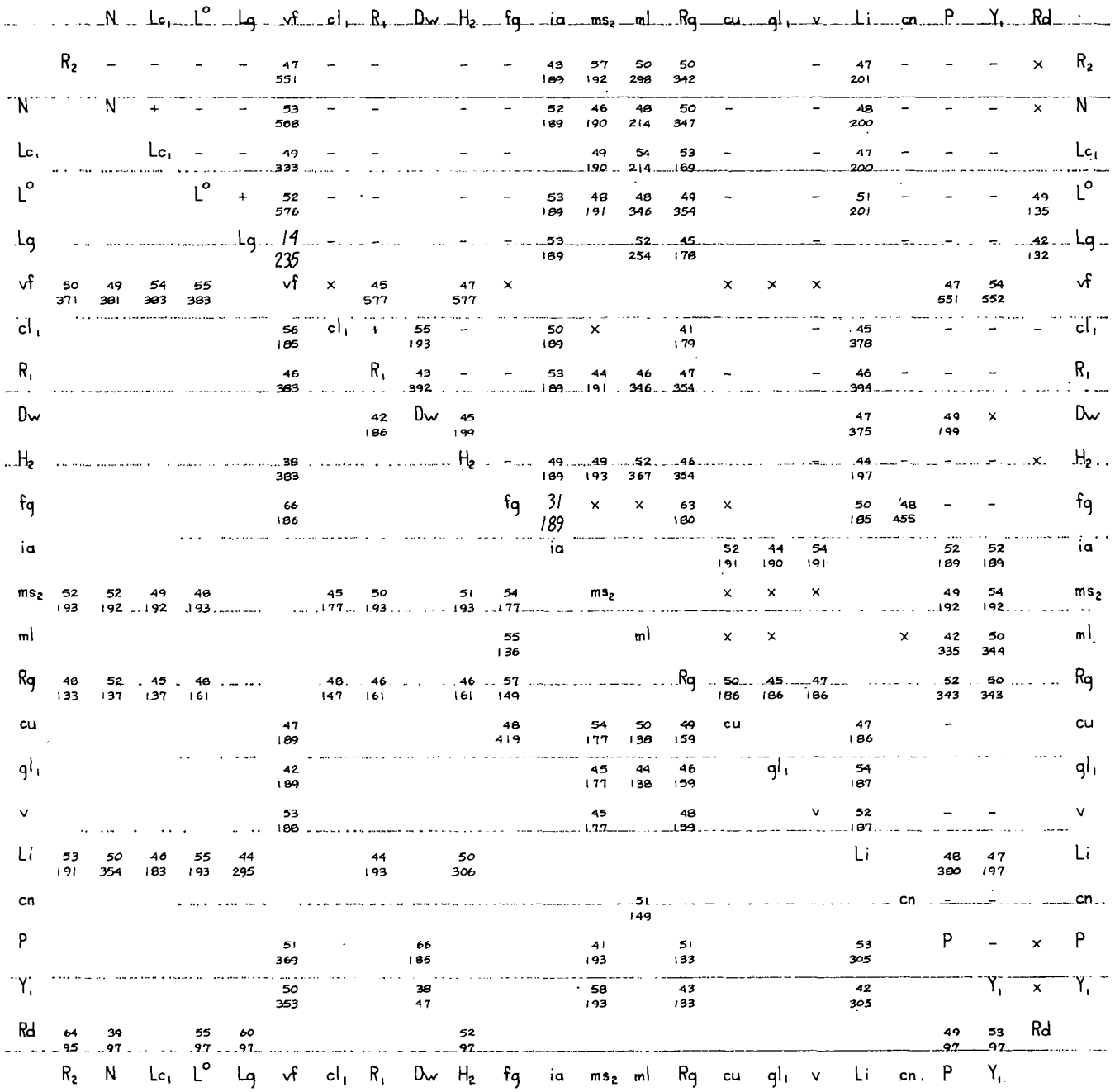

Figure 1. Summary of linkage tests (see text for explanation).

tains the F_2 population results. The numerical values are the previously unpublished results of linkage tests conducted at College Station, with the exception of two tests reported by Lewis, *cu-fg* (10) and *cn-fg* (11). In each cell the upper value is the recombination percent and the lower value is the population size. An (X) in a backcross cell indicates that the combination in question was tested only in the F_2. The character combinations reported by Stephens (20) are noted in Figure 1 by a dash or a plus sign to indicate independence or linkage, respectively. Refer to Stephens (20) for actual data on these combinations.

Several cases of suspected linkage were detected in preliminary experiments but subsequently these proved to be due to an inability to score one of the characters reliably in combination with certain others. The combined use of backcross and F_2 populations usually was adequate to resolve these problems. An example which was clarified through progeny tests was the *ml-P* pseudo-linkage. It was found that mosaic sectors in flowers with *ml ml* genotype made the classification of *P*-vs *pp* unreliable and resulted in pseudo-linkage values.

Linkage was detected between the *fg* and the *ia* loci. As a result of the tests with *fg, HA-2* was determined to be an allele of *fg* and *ia* proved to be linked with *fg. HA-2* has several gross phenotypically distinguishing characteristics which include a phenocopy of frego bract (15). Although the frego bract phenotype is one of the less severe and less striking manifestations of the *HA-2* pheno-

type, it is distinct and consistent enough that to test its relationship with *fg* was considered important. *HA-2* is dominant so that the F_1 with *fg* was frego bract in phenotype. The F_1 was backcrossed to *fg* and a 168 plant progeny was tested. The 88 *HA-2* segregates were classified as frego bract as were all 80 non-*HA-2* segregates. The lack of recombination demonstrates that *HA-2* and *fg* are part of a multiple allelic series.

The segregation of *fg* and *ia* is presented in Table 2. These mutants were linked by 30.7 units. In this same backcross population *cu* segregated and interacted with *ia* (7). There was no apparent influence of the interaction on the classification of *fg* and *ia* segregates, but in a similar F_2 population the interaction resulted in such distorted and unthrifty plants that scoring and classification of the frego bract expression proved to be impossible.

In Figure 1, *vf* is reported to be linked (13.6 units) with *Lg*. In addition to *vf*, *HA-1* and *rug* were also found to be linked with *Lg* by 8.3 and 10.7 units, respectively (Table 3). Tests of allelism of these three loci (Kohel, manuscript) suggest that these characters are either allelic or closely linked. The markers *vf* and *HA-1* originated independently as mutants in cultivated Upland cottons. Our records of rugose *(rug)* were incomplete until it was traced back to the notation Rugose Indore. Presumably, this character is Rugose Indore or Indore Crinkle found in commercial Upland cotton in India (4) and reported as the cr^I allele of the *cr* multiple allelic series (3). Therefore, *vf*, *HA-1* and *rug* must be part of the *cr* multiple allelic series. Stephens (20) reported linkage group II to L^o-41.2-*cr*-6.6-*Lg*. Unpublished data from this station have yielded larger values. The pooled data from backcross tests (446 plants) indicate that the map distances for linkage group II should be L^o-51.3-*cr*-11.6-*Lg*. Hutchinson (3) found the *cr*-*Lg* distance to be 10.2 units in a backcross population (305 plants).

The remaining new loci tested were independent of the loci with which they were tested. These loci will be tested in new combinations and they also will be used, along with the other independent loci, in the cytogenetics program for tests of association with established monosomes.

The *Dw* locus was included in these tests because it was reported by Rhyne (16) to be closely linked to R_1 (27.4 units). Rhyne studied interspecific hybrid material and the mutant allele was transferred from *G. raimondii*. The *Dw* we are using is a strong allele supplied to us by Rhyne who stated *Dw* became less closely linked to R_1 after recurrent backcrossing to *G. hirsutum*. In the initial population tested in our experiments, *Dw* was not significantly associated with R_1. To verify that we were still working with the *Dw* locus we used a monosome test. R_1 of linkage group III is located on chromosome 16 (White and Endrizzi, 22), and this monosome (Haplo-16) was used to test for association. This test gave positive proof that *Dw* was located on chromosome 16 and in linkage group III. The observed recombination of *Dw* and R_1 in all populations studied is 43 units.

A current summary of the results of cytogenetic tests of association between marker loci and chromosomes by the use of aneuploids has been presented by White and Endrizzi (22). Linkage groups II, III, and IV were placed on chromosomes 15, 16, and 6, respectively. In addition, L^L (Laciniate leaf) was placed on chromosome 1, and *ml* was placed on chromosome 4. These positive associations of marker loci and chromosomes are shown in Table 4. In addition, the new linkage group *fg*-*ia* reported in this study and the linkage of glandless leaf (gl_2) with withering-bract *(bw)* (17) are summarized in Table 4.

Table 2. Segregation and chi-square tests of the backcross *(Ialafgfg* × *iaiaFgFg)Ialafgfg.*

Segregation					Chi-square	
IaFg	Iafg	iaFg	iafg	T	Ia	1.51
27	72	59	31	189	Fg	0.43
RC = 30.7%					Linkage	28.20

Table 3. Segregation in linkage tests of *HA-1*, *vf*, and *rug* with *Lg* and chi-square tests of independence.

Segregation					Chi-square tests	
(hahaLgLg × HAHALgLg) hahaLgLg						
HALg	haLg	HALg	haLg	T	HA-1	0.89
3	29	37	3	72	Lg	0.89
RC = 8.3%					Linkage	50.00
(VfVfLgLg × vfvfLgLg) VfvfLgLg						
VfLg	vfLg	VfLg	vfLg	T	Vf	0.07
98	6	26	112	232	Lg	8.34
RC = 13.8%					Linkage	121.66
(nonrug-LgLg × rug-lgLg) rug-lgLg						
nonrug-Lg	rug-Lg	nonrug-lg	rug-lg	T	Rugose	0.11
60	8	7	65	140	Lg	0.71
RC = 10.7%					Linkage	86.43

Table 4. Summary of linkage groups in Upland cotton.

Linkage group	Chromosome	Marker loci
I	--	R_1-20-vg_2-32-Lc_1-44-N
II	15	L^o-51-*cr*-12-*Lg*
III	16	gl_1-17-R_1-43-*Dw*
IV	6	H_1-10-Lc_2
V	--	gl_2-17-*bw*
VI	--	*ia*-31-*fg*
	1	L^L
	4	*ml*

SUMMARY

The results of linkage investigations conducted over the past 20 years at College Station are reviewed. Nine new or untested mutants *(ia, ml, ms$_2$, Rg, vf, HA-1, HA-2, Li* and *rug)* were tested for linkage. The mutants *vf, HA-1,* and *rug* were linked with *Lg* and considered to be alleles in the *cr* multiple allelic series. The mutant *HA-2* was found to be an allele of the *fg* locus and *ia* was linked 30.7 units with the *fg* locus.

The genetic map distances in the linkage group L^o-*cr*-*Lg* were found to be greater than previously reported and the R_1-*Dw* recombination frequency has increased in a *hirsutum* background.

LITERATURE CITED

1. Brown, H. B., and Ware, J. O. Cotton (p. 131). McGraw and Hill Book Co., New York. p. 556. 1958.
2. Green, J. M. Frego bract, a genetic marker in Upland cotton. J. Hered. 46:252. 1955.
3. Hutchinson, J. B. The crinkled dwarf allelomoph series in the New World cottons. J. Genet. 47:178–207. 1946.
4. ———, and Ghose, R. L. M. On the occurrence of crinkled dwarf in *Gossypium hirsutum*. J. Genet. 34:437–446. 1937.
5. ———, and Silow, R. A. Gene symbols for use in cotton genetics. J. Hered. 30:461–464. 1939.
6. Kohel, R. J. Inheritance of accessory involucre mutant in American Upland cotton, *Gossypium hirsutum* L. Crop Sci. 5:119–120. 1965.
7. ———. Interaction of genes controlling accessory involucre and cup leaf mutants in cotton, *Gossypium hirsutum* L. Crop Sci. 5:158–159. 1965.
8. ———, and Lewis, C. F. Inheritance of ragged leaf mutant in American Upland cotton, *Gossypium hirsutum* L. Crop Sci. 2:61–62. 1962.

9. ————————. Inheritance of veins-fused mutant in American Upland cotton, *Gossypium hirsutum* L. Crop Sci. 2:174–175. 1962.
10. LEWIS, C. F. The inheritance of cup leaf in cotton. J. Hered. 45:127–128. 1954.
11. ————————. Interactions of genes for round leaf and frego bract in cotton. J. Hered. 48:169–171. 1957.
12. ————————. Genetic studies of a mosaic leaf mutant. J. Hered. 49:267–271. 1958.
13. MCMICHAEL, S. C. Occurrence of the dwarf-red character in Upland cotton. J. Agr. Res. 64:477–481. 1942.
14. ————————. Glandless boll in Upland cotton and its use in the study of natural crossing. Agron. J. 46:527–528. 1954.
15. MCNAMARA, H. C., and PORTER, D. D. Heritable abnormalities in cotton and their segregation ratios. J. Hered. 41:310–315. 1950.
16. RHYNE, C. L. Duplicated linkage groups in cotton. J. Hered. 48:59–62. 1957.
17. ————————. Inheritance of the glandless-leaf phenotype in Upland cotton. J. Hered. 53:115–123. 1962.
18. RICHMOND, T. R., and KOHEL, R. J. Analysis of a completely male-sterile character in American Upland cotton. Crop Sci. 1: 397–401. 1961.
19. SIMPSON, D. M. Fuzzy leaf in cotton and its association with short lint. J. Hered. 48:153–156. 1947.
20. STEPHENS, S. G. Linkage in Upland cotton. Genetics 40:903–917. 1955.
21. WARE, J. O.: Inheritance of lint colors in Upland cotton. J. Am. Soc. Agron. 24:550–562. 1932.
22. WHITE, T. G., and ENDRIZZI, J. E. Tests for the association of marker loci with chromosomes in *Gossypium hirsutum* L. by the use of aneuploids. Genetics 51:605–612. 1965.