RECOMBINATION BETWEEN SUPPOSEDLY HOMOLOGOUS CHROMOSOMES OF GOSSYPIUM BARBADENSE L. AND G. HIRSUTUM L.[1]

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HISTORICALLY, comparative cytological studies of related species are based on observations of meiotic pairing in interspecific hybrids. Counts are made on the numbers of paired bivalents at Metaphase I, polyvalent associations when present are recorded, and if the material is technically suitable these observations are supplemented by chiasmata counts in diplotene and metaphase associations. In genera such as Gossypium, in which many interspecific combinations can be studied, the cytological evidence so obtained provides a valuable and independent check on evolutionary relationships determined by classical taxonomic analysis.

Yet the phenomenon of preferential pairing in the polyploid derivatives of interspecific hybrids, first reported by NEWTON and PELLEW (1929), shows that the amount of bivalent pairing in an interspecific hybrid which contains only one genome from each parent is a crude measure, at best, of relative cytological affinity. In the colchicine derived tetraploid hybrid in which each parental genome occurs twice, pairing is competitive, and the relative amounts of homologous and heterologous association provide more refined measurements of intergenomic affinity. Some years ago, it occurred to the writer that relative pairing affinity could be expressed quantitatively by the appropriate use of genetic markers (STEPHENS 1950). GERSTEL and PHILLIPS successfully expanded this idea to develop a systematic study of several marker loci and applied it to the analysis of polyploids derived by colchicine treatment from many of the interspecific hybrids available in Gossypium (see GERSTEL and PHILLIPS 1958; also PHILLIPS 1961b for general summaries of their extensive survey). They were able to show that several of the American diploid species which exhibit similar meiotic pairing behavior (13 II + 13 I) in triploid hybrids with tetraploid *G. hirsutum*, give characteristically different amounts of preferential pairing in the corresponding hexaploid derivatives. Presumably these differences are related to degree of structural divergence between the chromosomes of similar but not homologous genomes. Slight, but significant amounts of preferential pairing have been found in the colchicine derived tetraploid hybrids of the Asiatic diploid species, *G. arboreum* L. and *G. herbaceum* L. (PHILLIPS 1961a). Evidence of

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preferential pairing in polyploid hybrid derivatives of *G. hirsutum* L. and *G. barbadense* L. is still lacking.

It had been suspected for some time that linked genes recombine less freely in segregating progenies of interspecific hybrids than they do in intraspecific segregations. This situation was noted by SILOW (1944) in the case of *arboreum* × *herbaceum* hybrids and was recently confirmed by PHILLIPS (1961a) in a carefully controlled test. Further, RHYNE (1958) showed that the insertion of a chromosomal segment from *arboreum* into a genetically marked and supposedly homologous region in *hirsutum* produced a change in linkage map distance. He pointed out that the effect might be due to general suppression of recombination in the affected chromosome or alternatively to a shift in chiasma position. These findings indicate that conditions which satisfy the requirements of normal pairing do not necessarily satisfy the requirements of normal recombination. It is perhaps justifiable to assume that the "recombination test" provides a more sensitive index of chromosomal homology than the "preferential pairing test," though its application is restricted to chromosomes marked with two or more loci.

The experiments to be described are concerned with a study of recombination between supposedly homologous chromosomes in *barbadense* and *hirsutum*. The segment within which recombination was studied is over 90 crossover units in length and includes five linked marker loci. Since in both species, chiasma frequencies at metaphase average around two per bivalent, it is likely that the marked segment includes most of the genetic map length of the chromosome. (For further discussion of this point, see STEPHENS 1955). The multiply marked segment allows recombination to be studied simultaneously in adjacent chromosomal regions, thus enabling the investigator to distinguish between general crossover suppression and shifts in crossover frequencies.

MATERIALS AND METHODS

The marked segment of the *hirsutum* chromosome which was studied in these experiments included the following loci:

N	— naked seed	n	— fuzzy seed
Lc_1	— brown lint	lc_1	— white lint
Yg_2	— normal green leaf	yg_2	— yellow-green leaf
R_2	— petal spot	r_2	— spotless

Their serial order and relative crossover distances had been determined previously (STEPHENS 1955). The linkage map is drawn to scale in Figure 1a.

Because of the normal bivalent pairing and fertility in *hirsutum* × *barbadense* hybrids it is usually assumed that all these loci are common to both species. Surprisingly enough, considering the attention which has been given to the comparative genetics of these species, the critical tests of allelism and/or location in a linkage group are only available for the R_2 locus (HARLAND 1929) and for the Yg_2 locus (RHYNE 1955). Homology in the other loci, N and Lc_1, is still only a reasonable assumption. Thus naked seeded mutants in *barbadense* are recessive,

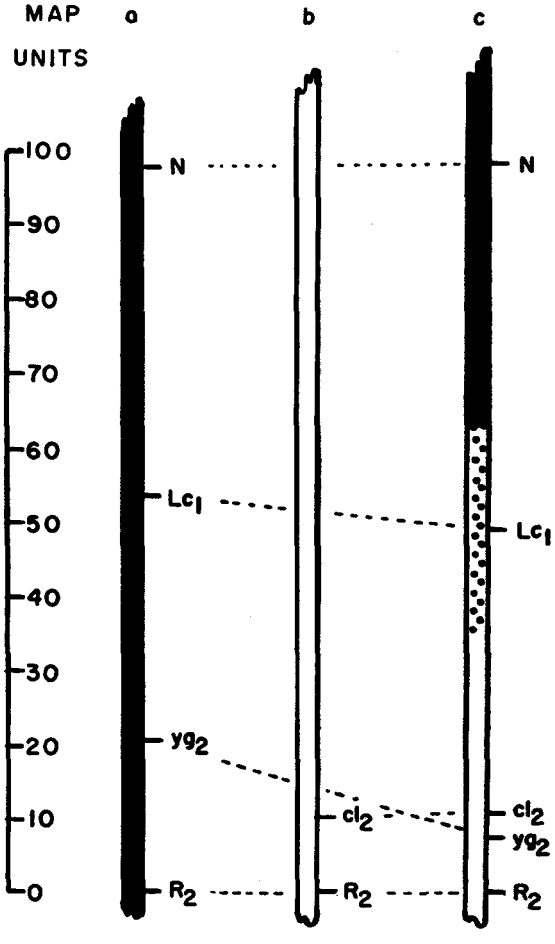

FIGURE 1.—Relative maps of supposedly homologous chromosomes of *barbadense* and *hirsutum* and their recombinant. (a) *hirsutum*, (b) *barbadense*, (c) recombinant obtained in the third backcross, *barbadense* to *hirsutum*. *Hirsutum* segments shown in black, *barbadense* in white, unknown origin, spotted.

not dominant as in *hirsutum*, and their comparative genetics are still obscure. No brown lint mutant at the Lc_1 locus has yet been found in *barbadense*.

Again, the recessive mutant, short-branch (cl_2) occurs in *barbadense*, and is known to be linked with R_2 with ten percent crossing over (SILOW 1946). No short-branch mutant has been found in *hirsutum* but it is usually assumed that this species carries a homologous locus with a stable normal allele, "Cl_2". On this assumption, the "Cl_2" locus in *hirsutum* should be placed either ten units below R_2 in the *hirsutum* map shown in Figure 1a, or else above R_2 and closely linked with Yg_2. From evidence to be presented later, it will be shown that the latter placement is correct. The linkage map of the supposedly homologous *barbadense* chromosome, on the basis of identical gene order, is shown in Figure 1b.

The crossing procedure which was adopted to analyze recombination within the marked chromosome is summarized in Figure 2. The primary *hirsutum* parent was an Upland marker stock, SM8, which carries the linked combination, N—Lc_1—"Cl_2"—yg_2—r_2. Phenotypically it has naked seeds, brown lint, normal fruiting habit, yellow-green leaves and spotless petals. The *barbadense* parent was an Egyptian strain, kindly provided by DR. S. O. S. DARK, Shambat Experiment Station, Sudan. Phenotypically, it has tufted (i.e. nonnaked) seeds, white lint, short-branch fruiting habit, normal green leaves and large petal spot. It should, therefore, carry the homologous linked combination, "n"—"lc_1"—cl_2—Yg_2—R_2. The F_1 was backcrossed to a multiple recessive *hirsutum* tester stock, SM4, having the linked combination, n—lc_1—"Cl_2"—yg_2—r_2. Although, theoretically, the recessive short-branch character should not have appeared in the first backcross generation, about one third of the plants exhibited the short-branch phenotype with varying degrees of penetrance. This suggested that cl_2, when transferred to a *hirsutum* background might show reversed dominance relationships in a manner previously described by HARLAND (1934) in the case of his "Crinkled Dwarf" transferences. It was planned, therefore, to select from the backcross the linked combination, N—Lc_1—cl_2—Yg_2—R_2, and to test recombination in a second backcross to SM4. The second backcross was made and the progeny raised before it was discovered that a white-linted (lc_1) selection had been made in error. This left the Lc_1 locus unmarked in the second backcross, but in any case the expression of the short-branch character was too mild and too variable for accurate scoring to be performed. Further selections were made in this

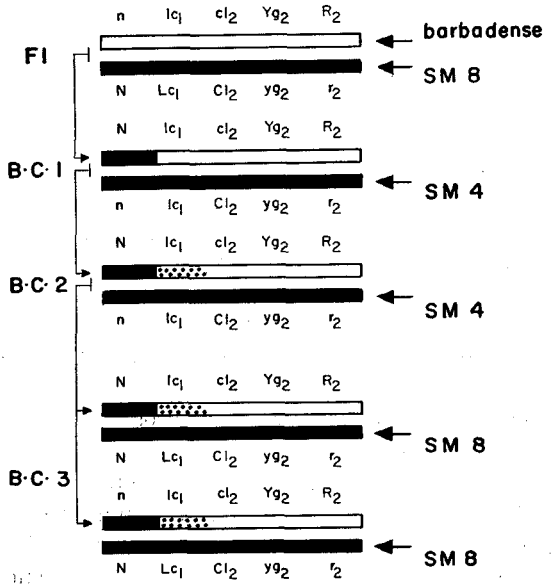

FIGURE 2.—Diagram of crossing program used to study recombination between supposedly homologous chromosomes of *barbadense* and *hirsutum*.

generation, therefore, for plants with well-expressed short-branch characteristics, normal green leaves and large petal spot. Some of the selections had naked, others fuzzy seeds. All were backcrossed to SM8, representing a third backcross to *hirsutum*.

Seven third-backcross families were grown from which five plants were selected for further study. Short-branch expression was still variable, both between and within families, indicating that dominance reversal was incomplete. Furthermore, there was a deficiency of short-branch segregates in all families. The five plants selected were all heterozygous for the *barbadense* segment, cl_2—Yg_2—R_2, and heterozygous for Lc_1. Three of the plants were heterozygous and two homozygous for N. The selfed families from these five selected plants provided the material for studies of recombination. Reference to the last two linkage diagrams in Figure 2, which represent the cytogenetic constitutions of the third backcross selections, shows that recombination in the interval, cl_2—R_2 would involve crossing over between *barbadense* and *hirsutum* segments (heterospecific recombination). Recombination in the interval between N and some position to the left of Lc_1 would involve exchanges between *hirsutum* segments only (homospecific recombination). Between these intervals there is a "no man's land" on either side of, and including, the Lc_1 locus. In this region recombination would involve exchanges between a *hirsutum* segment and a segment of unknown, and possibly mixed origin. This dubious region was a consequence of the mistake made in the selection of the parent for the second backcross, which has been noted earlier.

Because difficulty was anticipated in the scoring of short-branch, the selfed seeds from each of the five selections were divided into approximately equal lots, and duplicate families raised from them in successive seasons. This duplication under different seasonal conditions provided a check on scoring accuracy. As will be shown later, scoring proved to be reasonably consistent. The instability of *barbadense* petal spot, R_2, on transference to *hirsutum* created a more difficult problem and some loss of information. In the selfed progenies of the third backcross, somatic mutation was very common. It was expressed as an erratic reduction in size or complete disappearance of the petal spot in many R_2 segregates. An individual plant might produce varying proportions of spotted and spotless flowers; frequently the petals of a single flower might differ in presence or absence or size of spot. There was a general tendency for the proportion of spotless flowers to increase as the plants grew older, but early scoring did not solve the problem, because some plants which had been repeatedly scored as spotless would eventually produce a flower with a faint spot. It was necessary, therefore, to attempt daily scorings of each individual plant which had previously been scored as spotless, until a flower with a spot was observed. Only those plants in which a spotted petal had never been recorded were classified as spotless (r_2). In spite of the intensive scoring system used, the unstable families produced a large excess of apparently spotless segregates. It is probable that many R_2 segregates had mutated to r_2 before flowering commenced. Attempts to stabilize spot expression by selecting apparently stable R_2 plants were unsuccessful because somatic mutation re-

curred in their progenies. In the absence of counterselection, few spotted forms would have survived past the third backcross generation.

RESULTS

The five third-backcross selections were all heterozygous for the marked segment, Lc_1—"Cl_2"—Yg_2, and their selfed progenies provided information on recombination in this region. Only three of the five selections were appropriately marked in the N—Lc_1 segment, while in the yg_2—r_2 segment analysis was complicated by the instability of petal spot. For these reasons, it will be convenient to present the data on recombination from each of the three regions separately.

Recombination in the Lc_1—"Cl_2"—yg_2 *region:* The complete data for this region obtained in the seasons, 1959 and 1960, are presented in Table 1. Tests of homogeneity between seasons and between families were made. As shown at the right of the table, in none of the five families was there significant hetero-

TABLE 1

Segregations obtained in the selfed progenies of five selections from third backcross families (barbadense *to* hirsutum) *involving recombination in the* Lc_1–"Cl_2"—yg_2 *region. Dominant alleles (including* cl_2 *which shows reversed dominance in* hirsutum) *are indicated by* (+), *recessive alleles by* (—).

Family	Lc_1	+	—	+	—	+ *	— *	+	—	Total	$\chi^2(7)$	P
	cl_2	+	—	+	—	—	+	—	+			
	Yg_2	+	—	—	+	—	+	+	—			
S5580–39												
1959		71	5	2	1	29	32	4	1	145		
1960		57	5	1	2	17	35	3	0	120		
Total		128	10	3	3	46	67	7	1	265	4.4	0.70–0.80
S5582–7												
1959		96	4	3	0	31	31	4	0	169		
1960		61	4	0	1	23	22	1	0	112		
Total		157	8	3	1	54	53	5	0	281	4.8	0.50–0.70
S5582–16												
1959		87	7	3	1	28	40	2	3	171		
1960		61	8	1	0	13	34	0	1	118		
Total		148	15	4	1	41	74	2	4	289	6.0	0.50–0.70
S5582–36												
1959		71	4	3	0	30	50	3	1	162		
1960		67	0	1	1	11	26	3	0	109		
Total		138	4	4	1	41	76	6	1	271	13.8	0.05–0.10
S5582–38												
1959		93	5	1	0	21	35	5	0	160		
1960		38	4	1	0	15	21	3	0	82		
Total		131	9	2	0	36	56	8	0	242	3.3	0.80–0.90
GRAND TOTAL		702	46	16	6	218	326	28	6	1348		

Heterogeneity between families: $\chi^2(28) = 34.0$, P = 0.20–0.30.

* Parental classes starred.

geneity between the 1959 and 1960 scorings, though in the case of Family S5582-36 the chi-squared value is only slightly above the five percent level. Nor were there significant differences between families (see foot of table), so that it was permissible to calculate linkages from the grand totals.

The individual gene segregations and their paired linkage relationships are analyzed in Table 2. The former, shown at the top of the table deviate widely

TABLE 2

Analyses of individual gene segregations and linkage relationships in the Lc_1—"Cl_2"—yg_2 *region*
Data from Table 1

			Individual gene segregations		
			Total	$\chi^2(1)$	P
Expected (3 : 1)		1011:337	1348	...	.....
Lc_1:lc_1		964:384	1348	8.7	0.001–0.01
cl_2:"Cl_2"		1050:298	1348	6.0	0.01 –0.02
Yg_2:yg_2		1062:286	1348	10.3	0.001–0.01
			Linkages		
$Lc_1\ cl_2$	Lc_1 "Cl_2"*	$lc_1\ cl_2$*	lc_1 "Cl_2"	Total	C.O. percent
718	246	332	52	1348	38.8 ± 2.3
$cl_2\ Yg_2$*	$cl_2\ yg_2$	"Cl_2" Yg_2	"Cl_2" yg_2*		
1026	22	34	264	1348	4.5 ± 0.6
$Lc_1\ Yg_2$	$Lc_1\ yg_2$*	$lc_1\ Yg_2$*	$lc_1\ yg_2$		
730	234	332	52	1348	39.6 ± 2.3

* Parental classes starred.

from the expected Mendelian ratios. That these deviations are attributable to differential transmission or survival rather than inaccurate scoring is indicated by the fact that the deficient class is in each case associated with the allele introduced from the *hirsutum* parent. That is, the segment, Lc_1—"Cl_2"—yg_2, as a whole is deficient. The deficiency is satisfactorily explained by the weak growth and late flowering of all yg_2 types.

Crossover percentages were calculated both by the method of maximum likelihood and by the recombination method (see MATHER 1938). As the two methods yielded estimates which differed in all cases by less than two percent, the maximum likelihood estimate has been adopted throughout this study. The crossover values given in the lower part of Table 2 show that "Cl_2" and yg_2 are closely linked and that the probable map sequence is Lc_1—"Cl_2"—yg_2.

Recombination in the N—Lc_1 *region:* The complete data obtained from three families in the 1959 and 1960 seasons are presented in Table 3. Segregations obtained in the 1959 plantings were homogeneous, but in 1960 the duplicate plantings of the same families showed heterogeneity. Further analysis (see foot of Table 3) showed that the lack of agreement was chiefly due to the linkage component of heterogeneity. The individual gene segregations and linkage relationships are analyzed in Table 4. It is obvious that the 1960 plantings of Family

TABLE 3

Segregations obtained in the selfed progenies of three selections from third backcross families (barbadense *to* hirsutum) *involving recombination in the* N—Lc_1 *region*

Family	N^*Lc_1	$N\ lc_1$	$n\ Lc_1$	n^*lc_1	Total
1959					
S5580–39	75	24	31	15	145
S5582–16	92	40	28	11	171
S5582–36	73	37	34	18	162
Total	240	101	93	44	478
		$\chi^2(6) = 7.0$; P = 0.30–0.50			
1960					
S5580–39	66	33	12	9	120
S5582–16	50	33	25	10	118
S5582–36	70	17	12	10	109
Total	186	83	49	29	347
		$\chi^2(6) = 16.2$; P = 0.01–0.02			

Components of heterogeneity in 1960 season

Source	d.f.	χ^2	P
N vs. n	2	5.6	0.05–0.10
Lc_1 vs. lc_1	2	4.1	0.10–0.20
Linkage	2	6.5	0.02–0.05
Total	6	16.2	0.01–0.02

* Parental classes starred.

S5582-36 differ markedly from the others in linkage relationships. Excluding this planting, the crossover estimates do not differ significantly from independence—their combined estimate is 49.1 percent. The "aberrant planting" yields a significantly different estimate of 33.9 percent. No satisfactory explanation can be offered for this discrepancy, which involves an apparent difference in linkage relationships between duplicate plantings of the same family.

Recombination in the "Cl_2"—yg_2—r_2 *region:* In the third backcross, the expression of the short-branch character was too variable for accurate scoring, but four families were scored satisfactorily for leaf color and petal spot. It was in this generation that spot mutability first occasioned difficulty in scoring, and many of the plants scored as R_2 had become phenotypically r_2 by the end of the flowering period. However, as shown in Table 5, the individual gene segregations were in accordance with Mendelian expectation, the families were homogeneous, and a combined estimate of 8.2 percent crossovers was obtained.

Selfed families from five selected plants in the third backcross generation were grown in duplicate plantings in 1959 and 1960. The extent to which the somatic instability of petal spot would interfere with scoring was not appreciated in 1959 and no reliable estimates were obtained. In 1960, a system of daily scoring throughout the flowering period was initiated as explained earlier in this paper. In one of the five families, no petal spot appeared although its parent had been scored as R_2. In a second family, only ten percent of the plants produced flowers

with a faint and barely recognizable spot, and it was, therefore, useless for obtaining a linkage estimate. The remaining three families segregated as shown in Tables 6, 7 and 7a.

TABLE 4

Analyses of individual gene segregations and linkage in the N–Lc_1 *region. Data from Table 3*

Family	Individual gene segregations N:n		Total	$\chi^2(1)$	P	Lc_1:lc_1		Total	$\chi^2(1)$	P
1959										
Pooled data from three families	341	137	478	3.4	0.05–0.10	333	145	478	7.3	0.001–0.01
1960										
S5580–39	99	21	120	3.6	0.05–0.10	78	42	120	6.4	0.01 –0.02
S5582–16	83	35	118	1.4	0.20–0.30	75	43	118	8.2	0.001–0.01
S5582–36	87	22	109	1.4	0.20–0.30	82	27	109	0.0	0.90 –0.95

Family	Linkage N^*Lc_1	$N\ lc_1$	$n\ Lc_1$	n^*lc_1	Total	C.O. percent
1959						
Pooled data from three families	240	101	93	44	478	47.8 ± 3.4
1960						
S5580–39	66	33	12	9	120	47.7 ± 6.4
S5582–16	50	33	25	10	118	55.2 ± 7.4
S5582–36	70	17	12	10	109	33.9 ± 5.8
Joint estimate (excluding S5582–36 in 1960)	356	167	130	63	716	49.1 ± 2.1

* Parental classes starred.

TABLE 5

Segregations obtained in the third backcross, barbadense *to* hirsutum *involving recombination in the* yg_2–r_2 *region*

Families	Yg_2R_2*	Yg_2r_2	yg_2R_2	yg_2r_2*	Total
S5579	13	2	0	9	24
S5580	26	2	4	20	52
S5581	22	3	0	31	56
S5583	16	1	2	19	38
Total	77	8	6	79	170

$\chi^2(9) = 10.6$; P = 0.30–0.50
C.O. percent = 8.2 ± 2.1

	Individual gene segregations	Total	$\chi^2(1)$	P
Expected	85:85	170	..	...
Yg_2:yg_2	85:85	170	0.0	1.00
R_2:r_2	83:87	170	0.1	0.70–0.80

* Parental classes starred.

TABLE 6

Segregations obtained in the selfed progenies of three selections from third backcross families (barbadense *to* hirsutum) *involving recombination in the* "Cl_2"—yg_2—r_2 *region. Dominant alleles (including* cl_2 *which shows reversed dominance in* hirsutum *are indicated by* (+), *recessive alleles by* (—)

Family	cl_2 / Yg_2 / R_2	* + + +	* — — —	+ + —	— — +	+ — —	— + +	+ — +	— + —	Total
S5582–7		46	26	38	3	0	2	0	1	116
S5582–16		75	18	21	2	1	0	1	0	118
S5582–38		50	19	11	1	1	2	0	1	85
Total		171	63	70	6	2	4	1	2	319

$\chi^2(14) = 25.8$; P = 0.02–0.05

Components of heterogeneity

Source	d.f.	χ^2	P
cl_2:"Cl_2"	2	4.4	0.10 –0.20
Yg_2:yg_2	2	1.6	0.30 –0.50
R_2:r_2	2	13.1	0.001–0.01
Linkages	8	6.7	0.50 –0.70
Total	14	25.8	0.02 –0.05

* Parental classes starred.

TABLE 7

Analyses of linked segregations involving unstable R_2 *classes. Data from Table 6. The adjusted frequencies are given in parenthesis below each class. The adjustment factor,* d, *is estimated separately from each family as the proportion of* R_2 *types misclassified as* r_2. *For further information see text*

Family	"d"	* $Yg_2 R_2$	$Yg_2 r_2$	$yg_2 R_2$	* $yg_2 r_2$	Total	C.O. percent
S5582–7	0.414	48	39	3	26	116	.....
		(81.90)	(5.10)	(5.10)	(23.90)	...	9.2 ± 2.8
S5582–16	0.119	75	21	3	19	118	.....
		(82.69)	(5.81)	(5.81)	(23.69)	...	10.4 ± 3.0
S5582–38	0.169	52	12	1	20	85	.....
		(62.50)	(1.25)	(1.25)	(20.00)	..	3.0 ± 1.9
Total (unadjusted)		175	72	7	65	319	.....
(adjusted)		(227.09)	(12.16)	(12.16)	(67.59)		

Linkage heterogeneity between families: $\chi^2(2) = 3.8$; P = 0.10–0.20.

* Parental classes starred.

The data in Table 6 show that the three families gave heterogeneous segregations, and that the heterogeneity was almost entirely attributable to differences in the relative proportions of R_2 types recovered. It seems evident that despite the intensive scoring system adopted, a number of R_2 types had mutated prior to flowering and had, therefore, been misclassified as r_2. (As shown in Table 8, there

TABLE 7a

Analyses of linked segregations involving unstable R_2 *classes. Data from Table 6. The adjusted frequencies are given in parenthesis below each class. The adjustment factor,* d, *is estimated separately from each family as the proportion of* R_2 *types misclassified as* r_2. *For further information see text*

Family	"d"	* $cl_2 R_2$	$cl_2 r_2$	"Cl_2" R_2	* "Cl_2" r_2	Total	C.O. percent
S5582–7	0.414	46	38	5	27	116	.....
		(79.75)	(7.45)	(7.45)	(21.55)	...	13.8 ± 3.5
S5582–16	0.119	76	22	2	18	118	.....
		(83.81)	(4.69)	(4.69)	(24.81)	...	8.3 ± 2.7
S5582–38	0.169	50	12	3	20	85	.....
		(60.69)	(3.06)	(3.06)	(18.19)	..	7.5 ± 3.0
Total (unadjusted)		172	72	10	65	319	.....
(adjusted)		(224.05)	(15.20)	(15.20)	(64.55)		

Linkage heterogeneity between families: $\chi^2(2) = 2.4$; P = 0.30–0.50.

* Parental classes starred.

TABLE 8

Analyses of individual gene segregations and linkage in the "Cl_2"—yg_2—r_2 *region. Data from Tables 6 and 7. Adjusted classes in parenthesis*

Individual gene segregations			Total	$\chi^2(1)$	P
Expected (3:1)	239.25	:79.75	319	...	.....
cl_2:"Cl_2"	244	:75	319	0.4	0.50 –0.70
Yg_2:yg_2	247	:72	319	1.0	0.30 –0.50
R_2:r_2					
S5582–7	51	:65	116	59.6	0.001
S5582–16	78	:40	118	5.0	0.02 –0.05
S5582–38	53	:32	85	7.3	0.001–0.01

Linkages

* $cl_2 Yg_2$	$cl_2 yg_2$	"Cl_2" Yg_2	* "Cl_2" yg_2	Total	C.O. percent
241	3	6	69	319	3.0 ± 0.9
* $Yg_2 R_2$	$Yg_2 r_2$	$yg_2 R_2$	* $yg_2 r_2$	Total	
(227.09)	(12.16)	(12.16)	(67.59)	319	7.9 ± 1.6
* $cl_2 R_2$	$cl_2 r_2$	"Cl_2" R_2	* "Cl_2" r_2	Total	
(224.05)	(15.20)	(15.20)	(64.55)	319	10.0 ± 1.8

* Parental classes starred.

was significant deficiency of R_2 types in all three families.) If one makes the assumption that early somatic loss of spot is independent of recombination in the segment, "Cl_2"—yg_2—r_2, during the preceding meiosis, then the effects of misclassification may be corrected in the following manner:

Let p be the recombination fraction in the yg_2—r_2 interval. The expected proportions of the classes, $Yg_2\ R_2$, $Yg_2\ r_2$, $yg_2\ R_2$ and $yg_2\ r_2$ will be respectively,

$$\frac{2+P}{4}, \frac{1-P}{4}, \frac{1-P}{4}, \frac{P}{4}$$

$$\text{where } P = (1-p)^2$$

Let d be the proportion of R_2 types misclassified as r_2. Then the expected proportions of the four classes will become, respectively,

$$\frac{(2+\mathrm{P})(1-d)}{4}, \frac{1-\mathrm{P}+d(2+\mathrm{P})}{4}, \frac{(1-\mathrm{P})(1-d)}{4}, \frac{\mathrm{P}+d(1-\mathrm{P})}{4}$$

If e is the expected number of R_2 segregates in the family and o the number actually scored as R_2 then $d = \frac{e-o}{e}$ and can be estimated separately for each family.

The recombination fraction, p, can then be estimated using a modified form of the recombination method.

$$\frac{Yg_2R_2 \times yg_2r_2}{Yg_2r_2 \times yg_2R_2} = \frac{(2+P)(1-d)(P+d(1-P))}{(1-P+d(2+P))(1-P)(1-d)} =$$

$$\frac{(1-d)\ P^2 + (2-d)\ P + 2d}{(1-d)\ P^2 - (2+d)\ P + (1+2d)}$$

Using this method, the crossover percentages between Yg_2 and R_2 and between cl_2 and R_2 were calculated and are shown in Tables 7 and 7a respectively. Although the crossover estimates derived from the different families have a rather wide spread, they are not significantly different. This can be shown by recalculating the expected segregations for each family from its appropriate estimate of p, and so obtaining the adjusted frequencies which are shown in parenthesis in Tables 7 and 7a. Contingency tests can then be conducted on the adjusted frequencies. Since in fitting the data, two degrees of freedom are lost in the case of each individual gene segregation, the chi-squared value associated with the remaining two degrees of freedom is attributable solely to heterogeneity in the linkage component. In neither Table 7 nor 7a is this component significant, so that the adjusted frequencies of the individual families can be combined to provide estimates of crossover frequency. These are shown in the lower portion of Table 8.

The crossover estimates in Table 8 indicate that the map sequence is "Cl_2"—yg_2—r_2 which agrees with the sequence Lc_1—"Cl_2"—yg_2 obtained previously from the data in Table 2. The interval, "Cl_2"—yg_2 is common to both sequences, and the crossover estimates of 4.5 percent in Table 2 and 3.0 percent in Table 8 are in good agreement. Since the estimate obtained in Table 2 is based on five families and that in Table 8 on only a portion of the data from three of the same five families, the value of 4.5 percent may be regarded as the more reliable

estimate. The interval, yg_2—r_2, furnishes an estimate of 7.9 percent in good agreement with the backcross estimate of 8.2 percent in the previous generation (Table 5).

Principles of relative mapping: If two chromosomes from related species are strictly homologous, then, under comparable conditions, their patterns of recombination in hybrid condition should be exactly the same as those resulting from intraspecific recombination. Comparable conditions imply (1) a common cytoplasm (2) a similar genotypic background and (3) similar environmental conditions (temperature, nutrition, etc.) particularly during the meiotic process. If, under such conditions, patterns of interspecific and intraspecific recombination can be shown to be significantly different, the natural conclusion to be drawn is that homology between the chromosomes is imperfect.

Differences in recombinational patterns can be measured by relative mapping. Homologous chromosomes from the same species and heterozygous in a sequence of marker loci are testcrossed to provide data on which a standard linkage map may be constructed. A parallel testcross is performed involving supposedly homologous chromosomes from related species and marked as far as possible by the same loci. This yields a second or relative map which can be compared region by region with the standard map. Since from the early classical work with Drosophila it is known that recombination is reduced in non-homologous regions, and that in corn (unlike Drosophila) there is a concomitant increase in neighboring regions (MORGAN 1950; RUSSELL and BURNHAM 1950; RHOADES and DEMPSEY 1953), a systematic comparison of standard and relative maps should indicate rather precisely the location of any non-homologous segment. At the same time, a comparison of total map length enables a distinction to be made between an over-all reduction in recombination and a shift in crossover distribution.

The data on interspecific recombination reported here are comparable with data on intraspecific recombination published earlier (STEPHENS 1955). Both sets of data involve the same chromosomal region, *N* through r_2. In both cases the cytoplasmic background is that of the *hirsutum* marker stock, SM 4, since the SM 8 stock used in the present studies was synthesized from an intra-*hirsutum* cross which employed SM 4 as female parent. In the case of the intraspecific data, the genotypic background is pure *hirsutum*, as far as is known. All the markers used originated as mutants in standard *hirsutum* (upland) varieties. In the case of the interspecific data (a third backcross of *barbadense* to *hirsutum*) the genotypic background should average over 90 percent *hirsutum*. Minor differences in genotypic background do not have an important effect on recombination (RHYNE 1958). Finally, although the two series of data were collected in different seasons, the crosses and selfings were made in the same greenhouse during the winter months when attempts are made to preserve standardized growing conditions from season to season. While the effects of fluctuating temperature, nutrition, etc., cannot be discounted, it is unlikely from the practical point of view that these would differ more between seasons under winter green-

house conditions, than between plants growing in the same season under field conditions.

The data to be compared are shown in Table 9. Where backcross and F_2 seg-

TABLE 9

Relative maps of the N—Lc_1—yg_2—r_2 *region as determined from recombination between (A) homologous chromosomes of* hirsutum *and (B) supposedly homologous chromosomes of* hirsutum *and* barbadense. *Intra-*hirsutum *estimates calculated from data of* STEPHENS *(1955)*

Map interval	(A) Intra-*hirsutum* Estimated from	No.	C.O. percent	(B) *hirsutum* × *barbadense* Estimated from	No.	C.O. percent
N—Lc₁	Backcross	312	43.9 ± 2.8	F_2 (coupling)	716	49.1 ± 2.1
Lc₁—yg₂	Joint (B.C. + F_2)	169	33.2 ± 4.6	F_2 (repulsion)	1348	39.6 ± 2.3
yg₂—r₂	Joint (B.C. + F_2)	303	20.7 ± 2.7	Joint (B.C. + F_2)	489	8.0 ± 1.3
Total (*N—r₂*)			97.8 ± 6.0			96.7 ± 3.4

regations were both available, joint estimates of recombination have been calculated by the maximum likelihood method. Because the cl_2 marker was not available in the intraspecific data, it has been ignored in the interspecific data also, and map distances presented for the interval Lc_1—yg_2 (see Table 2). A comparison of the two sets of data shows that in the interval, yg_2—r_2, recombination has been reduced from 20.7 percent in the standard map to 8.0 percent in the relative map—a reduction of over 50 percent. Conversely, there has been a smaller *increase* in the other intervals, *N*—Lc_1 and Lc_1—yg_2. It will be recalled (Figure 2) that recombination in the yg_2—r_2 interval involves crossing over between *barbadense* and *hirsutum* segments while in the interval, *N*—Lc_1, only *hirsutum* segments are concerned. Recombination has, therefore, been reduced in the heterospecific region and compensated by increased recombination in the homospecific region. As a consequence, recombination over the total map length remains virtually unchanged (97.8—98.7 percent). The most reasonable interpretation of these findings is that the *barbadense* and *hirsutum* chromosomes are not completely homologous in the yg_2—r_2 region and, as a consequence, recombination is blocked over a portion of this interval. Owing to a compensatory increase in recombination elsewhere, the total recombination over the chromosome as a whole remains unchanged. *If this situation proves to be a common one in interspecific hybrids, it would indicate that the standard methods of counting chiasma frequencies per bivalent may sometimes provide quite misleading information on chromosomal homologies.*

In Figure 1c the relative map of the chromosome has been drawn to scale. This differs from the data presented in Table 9 in the fact that information on the cl_2—yg_2 interval is included in the map. It can be seen by comparing the standard *barbadense* map (Figure 1b) with the relative map (Figure 1c) that the distance between cl_2 and R_2 is practically unchanged. Assuming the same relative positions of cl_2 and yg_2, this interval is much longer in the standard *hirsutum* map (Figure 1a). This indicates that most of the interval, cl_2—R_2, in the *barba-*

dense chromosome has a homologous segment in its *hirsutum* counterpart, but the latter contains an extra segment not currently mapped in *barbadense*. Otherwise, the relative map should be shorter than either standard map in this region. The nature of the extra segment is at present unknown—it could represent the insertion of a segment from another chromosome (translocation), a duplication, or an inversion including R_2 and an unmapped region distal to it.

The material studied so far has only permitted relative mapping to be carried out in the heterospecific cl_2—R_2 end of the chromosome under investigation. With the stocks now available, one should be able to apply a similar analytical method to the N—Lc_1 region and, possibly, through interference analysis to locate the centromere. (It is known from earlier studies (STEPHENS 1955) that interference phenomena can be detected over long intervals in this chromosome and may be determined more by an inherently low chiasma frequency than by physical map distance.)

DISCUSSION

Compensatory recombination: The decrease in recombination observed in the yg_2—r_2 region was accompanied by increases over the remaining segments of the total mapped region of the chromosome. The net effect of these negatively correlated changes was to leave the total map length of the chromosome unaffected. This suggests that the total recombination in this particular chromosome is under genetic control and that a decrease in one region may be rather exactly compensated by an increase elsewhere. This apparent compensation might be considered fortuitous were it not for the fact that in corn the data of MORGAN (1950); RUSSELL and BURNHAM (1950); and RHOADES and DEMPSEY (1953) lend themselves to a similar interpretation. These investigators studied independently a situation in which recombination in a marked chromosomal region, with or without an included inversion, could be compared. Two inversions were paracentric, one pericentric, and the map length in which they were included varied from 16 to 42 crossover units. Within each specified region, recombination in inversion heterozygotes was virtually the same as that found in structural homozygotes. Suppression of effective crossing over within the inversion loops was almost exactly compensated by increased recombination in the regions *proximal* to the inversions. In the *distal* regions recombination remained unchanged. Compensation restricted to the proximal regions may be inferred also in the case of the cotton material because the chromosomal map indicates that the non-homologous region is terminal or subterminal in position.

These data as a whole support the hypothesis that total recombination frequency *per bivalent* may be under quite stringent genetic control, though the positions where recombinations occur may be quite labile and influenced by local structure. The concept of a genetically limited recombination frequency is not new (DARLINGTON 1939) and has been incorporated into current understanding of the evolution of breeding systems under natural selection (STEBBINS 1950; GRANT 1958). The concept is also in accordance with the conservatism in map

length which is exhibited by most genetic material which has been studied adequately. As far as I am aware no published map length, save that of the sex chromosome in *Habrobracon juglandis*, is significantly in excess of 150 crossover units. This indicates that over a wide range of material the modal number of recombinations is restricted within the narrow range of 1–3 per bivalent. A similar conclusion may be reached from the masses of data which have been published on cytological counts of chiasma frequency. Since the map lengths in different species are quite unrelated to physical length, there would seem to be no plausible mechanical significance for the restriction. That it is an optimum restriction, fixed by natural selection and genetically determined, would seem to be a logical assumption. As a corollary, one might expect that mechanisms are available for buffering it against the effects of structural aberrations. The intrachromosomal compensation suggested here may provide one such mechanism—the interchromosomal compensation in Drosophila, another (SCHULTZ and REDFIELD 1951). Possibly the two mechanisms are alternative and of different relative importance in different genetic systems.

Two further points of interest are worth comment. In the corn data, compensation appears to be achieved in the immediate neighborhood of the non-homologous region—in cotton the compensation is spread over the remainder of the mapped region. There is no reason to expect that the pattern of adjustment—if such it is—would be identical in two unrelated species, but judging by their map lengths the corn chromosomes (2 and 3) have a greater chiasma frequency than is found in the cotton chromosome. The second point of interest is that in the corn data, the compensatory mechanism apparently applies only to the homologous regions, i.e. the crossover estimates do not include the genetically ineffective recombinations *within* the inversion loops. Nor is there compensation *distal* to the inverted regions. This may indicate that the compensatory mechanism is blocked by a break in the normal chromatin sequence, just as interference phenomena may be blocked by the interposition of a centromere.

Cryptic structural differentiation: In 1950, it was suggested that cytological, genetic and breeding behavior of fertile interspecific hybrids in Gossypium was not sufficiently explained by HARLAND's former theory (1936) of multiple gene substitution and that cryptic structural differences had developed with speciation. Both these concepts have become outdated with time and what was once controversial should become a dead issue. In current terms the simplest form of "mutation" probably involves a structural rearrangement i.e. a change in the order of base pairs in a nucleotide sequence. Most likely all fundamental genetic changes are "structural" at the molecular level and "cryptic" under the light microscope. From the data presented here, however, it is clear that normal meiotic pairing resulting in fertile progeny is not a reliable criterion of chromosomal homology. *There is a level of chromosomal differentiation between species which can be detected only by special techniques (e.g. preferential pairing, relative mapping) but which may have quite important consequences in plant breeding.* For instance, it would be unrealistic for a plant breeder to assume that the progeny of a fertile interspecific hybrid would segregate and recombine as freely as

an intervarietal cross. Depending on the specific patterns of chromosomal differentiation in the parental species, shifts in chiasma position and frequency could generate an unexpected amount of recombinational variation in some parts of the genome and completely block recombination elsewhere. New techniques are needed to make efficient use of interspecific hybrids (STEPHENS 1961a,b).

The precise nature of the non-homologous segment which differentiates the "homologous" chromosomes of *barbadense* and *hirsutum* reported in this paper remains unknown. It is probably significant that the R_2 (spot) locus associated with the region, though stable in its own species, becomes somatically unstable in interspecific combination. It is possible that an inversion is involved and that the somatic instability may result from a breakage-fusion-bridge cycle similar to that found in corn by MCCLINTOCK (1941). This possibility is under investigation. A second interesting feature is the reversal of dominance at the cl_2 locus. In Gossypium as a whole, dominance reversal has only been found in interspecific crosses and in all cases studied the reversal is only manifested in mutant loci which are "species-specific" (i.e. the mutant occurs in one parental species and is unknown in the other). The common assumption that the species in which the mutant has never occurred carries a normal allele at the same locus is only an assumption and needs experimental verification.

SUMMARY

Two supposedly homologous chromosomes of *Gossypium barbadense* L. and *G. hirsutum* L. are shown to differ by a non-homologous terminal segment. In the non-homologous region crossing over is reduced from 20 to eight percent, but the reduction is compensated by slight but general increases in other marked segments of the chromosome. As a result, total map length remains virtually unchanged. The compensatory mechanism appears to be similar to that found in corn, where the reduction of crossing over in inverted regions is accompanied by increases in the immediately proximal regions. Cotton differs from corn in the fact that "compensation" is absorbed over most of the chromosomal arm and is not restricted to the immediately proximal region. It is pointed out that somatic instability and reversal of dominance in neighboring loci appear to be associated in some way with the lack of homology.

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