THE CYTOGENETICS OF SPECIATION IN ASIATIC COTTON¹

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THE cultivated cottons are divided into two groups: the diploid Asiatic species *Gossypium arboreum* and *G. herbaceum* and the New World amphidiploids *G. hirsutum* and *G. barbadense*. The taxa within each of these groups are readily crossed, and the F_1 in each case is vigorous and fertile. F_2 and subsequent generations, however, contain either depauperate types or plants that closely resemble one of the parents.

HARLAND (1936), after considerable study on the genetics of *G. hirsutum* and *G. barbadense*, concluded that the breakdown following hybridization of these species was due to the rearrangement of their coadapted modifier systems.

SILOW (1944a) employed much the same argument to account for the specific differentiation between *G. arboreum* and *G. herbaceum*, and rejected any explanation based on chromosome structural differences between the two species.

STEPHENS, in a 1950 review citing genetic, cytological, and plant breeding evidence, indicated that multiple gene substitution alone cannot account for the differentiation in these species. He suggested that species of *Gossypium* differ from one another by many chromosome structural differences too small to be easily detected by cytological methods. He further set forth three cytogenetical criteria by which cotton species could be tested for this cryptic structural differentiation: (1.) Selective elimination of donor-parent genotype in interspecific backcrosses. (2.) A reduction in crossing over in interspecific crosses. (3.) Deviation from autopolyploid segregation ratios in synthetic polyploids of interspecific hybrids.

STEPHENS (1949) and LEWIS and MCFARLAND (1952) in separate studies that complemented each other demonstrated selective elimination of donor-parent genes in backcrosses of *G. hirsutum* $\times$ *barbadense* hybrids. LODEN (1950) previously had shown that recombination for the red (R_1)-cluster (Cl_1) linkage is less frequent in the *G. hirsutum* $\times$ *barbadense* hybrid than in either parent.

The present study was designed to test all three of STEPHENS' criteria in a series of crosses involving the two Asiatic diploid species *G. arboreum* and *G. herbaceum*. The genetic marker stocks used in these crosses and the alleles they carry are listed in Table 1.

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TABLE 1
Genetic constitution of marker stocks

<i>G. arboreum</i>				<i>G. herbaceum</i>	
SMA1			S5001	"red" <i>herbaceum</i>	
R_2^{mgs}	Red plant body	r_2	Green plant body	R_2	Red plant body
F	Tufted seed	f	Fuzzy seed	f	Fuzzy seed
$*L^N$	Narrow leaf	l	Broad leaf	l	Broad leaf
$*cu$	Curly leaf	Cu	Non curly leaf	Cu	Non curly leaf
$*Lc_1$	Brown lint	lc_1	White lint	lc_1	White lint

* Linked loci.

TABLE 2
Backcross segregations of intraspecific G. arboreum and interspecific G. arboreum × herbaceum F₁ hybrids

P	χ^2	Intraspecific SMA1 × S5001 Seg.	Marker*	Seg.	Interspecific SMA1 × red <i>herb.</i> χ^2	P
.8-.9	.039	112	r_2 or R_2	228	.307	.5 -.7
		115	R_2^{mgs}	240		
		115	l	225		
.9-.95	.004	114	L^N	243	.6922	.3 -.5
		118	Cu	220		
.5-.7	.214	111	cu	248	1.675	.2 -.3
		122	lc_1	231		
.3-.5	.982	107	Lc_1	237	.077	.8 -.9
		124	fz	208		
.1-.2	1.942	103	Fz	260	5.778	.01-.02

* The *G. herbaceum* allele is listed first for each segregation.

MATERIALS AND METHODS

From the plant materials listed in Table 1 the intraspecific *G. arboreum* (SMA1 × S5001) and interspecific *G. arboreum* × *herbaceum* (SMA1 × "red" *herbaceum*) crosses were made. Upon being crossed with a suitable recessive *G. arboreum* tester stock they gave the data on gene transmission and crossing over recorded in Tables 2 and 3. Segregation frequencies from 4x intraspecific, inter-strain *G. arboreum* and 4x (*G. arboreum* × *herbaceum*) are recorded in Table 4. The synthetic amphidiploids were made by using the colchicine-tragacanth method of SCHWANITZ (1949). These tetraploids are duplex for the marker genes

TABLE 3

Linkage between L^N , Cu and Lc_1 in intraspecific G. arboreum and interspecific G. arboreum $\times$ herbaceum F_1 hybrids

Intraspecific SMA1 $\times$ S5001 Percent c.o.	No.	Crossover class*	Interspecific SMA1 $\times$ red <i>herb.</i> No.	Percent c.o.
		Parental		
	72	<i>L-cu-Lc</i>	184	
	78	<i>l-Cu-lc</i>	172	
65.5	150		356	76.1
		Singles		
	12	<i>l-cu-Lc</i>	24	
	17	<i>L-Cu-lc</i>	19	
12.6	29		43	9.2
		Singles		
	23	<i>L-cu-lc</i>	39	
	21	<i>l-Cu-Lc</i>	28	
19.2	44		67	14.3
		Doubles		
	4	<i>l-cu-lc</i>	1	
	2	<i>L-Cu-Lc</i>	1	
2.6	6		2	0.4
Heterogeneity $\chi^2 = 26.69$, d.f. = 3; $P = < .01$				

* Gene symbols abbreviated.

TABLE 4

Tetraploid segregation in synthetic polyploids of interspecific G. arboreum and interspecific G. arboreum $\times$ herbaceum F_1 hybrids

Combination	Anthocyanin pigmentation			$L^N L^N$	$L^N l$	Leaf-shape			ll
	$R_2 R_2$	$R_2 r_2$	$r_2 r_2$			ll	$L^L L^L$	$L^L l$	
Intraspecific									
4x(SMA1-S5001) $\times$ Upland	33	156	42	145*	30	..	..	..	..
4x(S5133-S5001) $\times$ Upland	..	..	..	..	..	..	52	201	46
χ^2 (1:4:1 or 5:1)	1.129			.026		.399			
P	(2)	.5-.7		(1)	.8-.9	(2)	.8-.9		
Interspecific									
4x(SMA1-red <i>herb.</i>) $\times$ Upland	76	308	67	406*	46	..	..	..	..
4x(S5133-red <i>herb.</i>) $\times$ Upland	..	..	..	..	..	..	47	418	56
χ^2 (1:4:1 or 5:1)	1.282			13.678		43.525			
P	(2)	.5-.7		(1)	<.01	(2)	<.01		

* The Narrow ($L^N L^N$) and intermediate ($L^N l$) leaf-shape classes were indistinguishable; hence leaf-shape was scored as a two-class segregation.

listed in Table 1 and are therefore expected to segregate 5:1 or 1:4:1 for the markers if random chromosome pairing and disjunction are assumed. Preferential pairing will decrease segregation (widen ratio); chromatid segregation will tend to increase it.

Ideally the intraspecific and interspecific hybrids should have been tested with both *G. arboreum* and *G. herbaceum* stocks, but the suitable recessive alleles were available only in *G. arboreum*; hence the backcross was made in only one direction.

RESULTS

Gene transmission in diploid $F_1 \times G. arboreum$ tester: As can be seen in Table 2 only one of the segregations studied deviated significantly from the expected 1:1 backcross ratio. In this, the segregation for amount and distribution of seed fuzz, there was considerable elimination of the *G. herbaceum* factor (*f*). In the remaining four segregating families there was a nonsignificant discrimination against gametic transmission and/or zygotic survival of *G. herbaceum* genes.

Genetic recombination in intra- and interspecific F_1 hybrids: Genetic recombination within the three point linkage group delimited by the leaf shape and lint color loci and including the curly locus was significantly reduced in the interspecific hybrid as compared with the intraspecific F_1 . Thus crossing over between L^N and Lc_1 was 37.0 percent in the intra-*arboreum* cross and reduced to 24.3 percent in the interspecific hybrid. SILOW (1944b) found a reduction in crossing over between leaf shape and lint color of a similar magnitude in interspecific hybrids of *G. arboreum* and *G. herbaceum* as compared to their parents.

Segregation in tetraploid $G. arboreum \times herbaceum$: Segregating progenies from the cross 4x (*G. arboreum* SMA1 $\times$ "red" *herbaceum*) $\times$ commercial Upland were scored for leaf shape and anthocyanin pigmentation (Table 4). The remaining markers could not be scored because of lack of a suitable tetraploid tester. Segregation for R_2 was 384 red:67 green (5.31:1), very close to the autotetraploid 5:1 ratio. The segregation for leaf shape was significantly different from that for anthocyanin coloration, being 406 narrow:46 broad or 8.83:1. The segregations for these two markers from *G. arboreum* $\times$ *herbaceum* amphidiploids, along with ratios for three other markers, have been reported previously by GERSTEL and PHILLIPS (1957). Four of the five segregations recorded in that report were near the autotetraploid ratio (5:1 or 1:4:1) while the fifth, that for leaf shape, was 9.8:1 (49 narrow:5 broad). Because of the small population scored for leaf shape this deviation from a 5:1 ratio was not significant. The present segregation of 8.83:1 based on a population of 452 plants is different and, as will be brought out below, indicative of chromosome structural divergence of *G. arboreum* and *G. herbaceum*.

DISCUSSION

Divergence between *G. arboreum* and *G. herbaceum* for the chromosome segment containing the leaf-shape, curly and lint-color loci is indicated by all three

of the criteria applied. The test for gene transmission is less decisive than the remaining two since the differences here, though indicating a trend toward donor elimination for all three loci, were nonsignificant. The deviations for genetic recombination and tetraploid segregation were significant.

A decrease in crossing over in an interspecific hybrid can be explained on a structural basis, i.e., less chromosome homology will result in reduction of chiasmata, or on the assumption that gametic or zygotic selection eliminates many of the non-coadaptive gene arrangements effected by crossing over. But if this latter explanation was applicable, one would expect that gametic elimination would be proportional to the total chromosome length (and therefore number of genes) involved in recombination (i.e., a product of meiosis in which 25 percent of the total chromosome length was involved in recombination events would be more subject to elimination than a meiotic product in which only 5 percent of the total chromosome length was affected by crossing over). If depression in over-all recombination percentage is a result of gametic selection and the foregoing assumptions are valid one would expect that the percentage reduction in recombination from control to the interspecific hybrid would be greater between the proximal locus (gene order, relative to centromere, is not known) of the linkage group and *Cu* than between the distal locus and *Cu*; this being due to greater survival value of gametes with a lesser amount of recombined chromatin. That this is not the case is indicated by the fact that in the interspecific hybrid the reduction in crossing over between *L* and *Cu* (27%) was very similar to the reduction between *Cu* and *Lc* (26%). Further, interference is negligible in the intraspecific cross (coinc. = 1.1) and significant in the interspecific cross (coinc. = .33). Since it is unlikely that selective elimination would influence interference (double crossovers would involve no more, and probably less, chromatin than singles) this is additional evidence that a reduction in chiasmata frequency is responsible for the observed decrease in recombination.

Since this test of the relative importance of gametic elimination in accounting for observed gene frequencies in interspecific backcrosses and F_2 's involves something less than five percent of the total chromosome length, it cannot be considered a particularly critical method of evaluation. The data (especially those for double crossovers) do indicate, however, that selective gametic elimination is not a major factor in the reduction of genetic recombination in the interspecific hybrid.

An explanation attributing reduction in crossing over in the interspecific hybrid to reduction in chiasmata frequency between incompletely homologous chromosomes seems to fit the data better than does any other explanation. The significant decrease in single and double crossovers would be expected and predictable on this basis.

The tetraploid segregation for L^N was scored in 1958. Since the 8.83:1 ratio for this allele was quite different from the segregations scored for other loci in synthetic amphidiploids of the Asiatic species (which clustered around 5:1) it seemed desirable to verify this value by studying the tetraploid segregation of another leaf-shape allele (L^L) of the Asiatic leaf-shape series. It was also deemed desirable to determine whether the leaf-shape locus was on one of the chromosomes for

which *G. arboreum* and *G. herbaceum* differ in end arrangement (GERSTEL 1953). The segregation of L^L (9.12:1, Table 4) proved to be very similar to that for L^N . There was no correlation between the leaf-shape locus and the semisterility or the cytologically detectable quadrivalent associated with the *G. arboreum-herbaceum* translocation.

The 8.98:1 segregation for leaf-shape (L^N and L^L segregations combined) must be the result of preferential pairing brought about by chromosome structural differences. It is improbable that genic differences could result in such a significant deviation from the autotetraploid 5:1 ratio.

In recent years several investigators (MICHIE 1953; WALLACE 1953 and 1958) have evolved the "affinity" concept to explain the apparent linkage of genes known to be independent. Thus loci can exhibit "quasi-linkage" due to differential affinity of centromeres resulting in their non random assortment to the poles at metaphase I. Such a mechanism could account, at least in part, for the preponderance of parental type recovered in backcrosses, F_2 and F_3 progenies of *G. arboreum* $\times$ *herbaceum* hybrids. There is no way of determining from the present data the importance of affinity relative to the elimination of gametes and zygotes imbalanced by non-coadaptive chromosomal arrangements, though the presence of depauperate types in early generation *G. arboreum* $\times$ *herbaceum* progenies would seem to indicate that the later phenomenon is of some importance: the possible role of affinity remains a moot question.

An interesting parallel to the situation described above for *G. arboreum* $\times$ *herbaceum* hybrids is found in early-generation progenies of *G. arboreum* or *G. herbaceum* $\times$ *anomalum*. Here sterility is greater and F_2 breakdown more drastic. The cytological differences in either of these crosses are quite obvious rather than "cryptic" as in the *G. arboreum* $\times$ *herbaceum* hybrid. An average of nine bivalents are seen in F_1 *G. arboreum* $\times$ *anomalum* or *G. herbaceum* $\times$ *anomalum* (SKOVSTED 1937), and tetraploid segregation for several markers average 50:1 (GERSTEL and PHILLIPS 1957). As the data presented in this paper indicate, and as STEPHENS (1950) has pointed out, there is every reason to assume that the differences between *G. arboreum* and *G. herbaceum* on the one hand and between the Asiatics and *G. anomalum* on the other differ in degree rather than kind.

SUMMARY

The degree and nature of speciation in the Asiatic cottons, *G. arboreum* and *G. herbaceum* have been determined by assessing the genetic behavior of the linked loci L , Cu , and Lc_1 and two other loci in the light of gene transmission, genetic recombination, and tetraploid segregation in their interspecific hybrids.

A nonsignificant degree of selective elimination of donor parent genotype in backcross progenies was noted for each of the linked loci. There was a significant amount of elimination found for one of the two other loci.

Crossing over within the L - Cu - Lc_1 linkage and segregation for leaf shape were both significantly altered from expected in the interspecific hybrid.

The results are explained on the basis of cryptic structural differentiation of the chromosomes of the two species.

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