THE COMPARATIVE GENETICS OF GOSSYPIUM HIRSUTUM L.
AND THE SYNTHETIC AMPHIPLOID, GOSSYPIUM
ARBOREUM L. $\times$ GOSSYPIUM THURBERI TOD.¹

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THE varieties of the cultivated New World species of *Gossypium* have been selected for a number of years with respect to certain economic characteristics. The intense pressures imposed by new demands of manufacturers, development of diseases, cultural methods, cost of production and other factors require wide adaptability of the breeding materials. Selection for these factors is often difficult because of the lack of variability presently available. This lack of variability is presumably due to the rather limited ancestry from which each variety originated. Related species comprise a potential source of such variability. The question arises then as to how useful these species may be for the breeder. To evaluate a given character as desirable in pure species alone is misleading because its usefulness is based upon the feasibility of transferring it into the species to be improved. Detailed studies of the nature of species differentiation are necessary to make possible the use of species hybrids as a source of breeding material. It is important therefore to develop methods of measuring differences between chromosomes of the cultivated amphiploid species and the chromosomes of related species. The use of "marker" genes, located in as many chromosomes as possible, facilitates the study of the transmission of genes from hybrid to progeny.

The present work constitutes a comparative genetic study of *Gossypium hirsutum* L., a cultivated amphiploid species, and its related diploid species, *G. arboreum* L. and *G. thurberi* Tod. Cytological studies have detected little differentiation between the chromosomes of *G. thurberi* and the D subgenome of *G. hirsutum* (BEASLEY 1942). The *arboreum* genome differs from the A subgenome of *hirsutum* by three reciprocal translocations (GERSTEL 1953; GERSTEL and SARVELLA 1956), but otherwise pairing seems to be regular. Nevertheless, it has been suggested (STEPHENS 1950) that more differentiation exists than is apparent from the cytological observations and that cryptic structural differences exist which are below the resolving power of the microscope. Cryptic structural differences could include not only small scale rearrangements of essentially the same loci, such as inversions and segmental interchanges, but also changes in the

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number and kind of loci resulting from deletions and nonreciprocal translocations. The preferential pairing of chromosomes in interspecific hybrids (IYENGAR 1942; GERSTEL and PHILLIPS 1958; PHILLIPS 1961b) and the reduction of recombination between linked genes in interspecific crosses (RHYNE 1958; PHILLIPS 1961a; STEPHENS 1961) are lines of evidence which support the hypothesis of cryptic differentiation but do not prove it. Furthermore, their results are equally compatible either with rearrangements *per se* or with deficiencies resulting from structural changes. However, if the latter situation contributes to chromosomal differentiation, one might expect to find cases in which mutant loci in one species would not be represented by their normal alleles in another related species. Testing this possibility is the main subject of the present work.

Crosses between *G. arboreum* "marker" stocks with *G. thurberi* and their corresponding synthetic amphiploids were produced. These amphiploids were crossed to *hirsutum* stocks and testcrossed to recessive *hirsutum* testers. It was hoped to obtain from the segregations the following information: (1) Determination of whether recessive mutants in *hirsutum* have dominant alleles in *arboreum*, or in *thurberi* or in both diploid species. (2) The location of *hirsutum* loci in the corresponding A or D subgenome.

MATERIALS AND METHODS

Materials: The species of *Gossypium* used in this investigation are the following:

- (1) *G. arboreum*—Cultivated Asiatic species with $n = 13$ chromosomes.
(A₂ genome)
- (2) *G. thurberi*—Wild diploid American species with $n = 13$ chromosomes.
(D₁ genome)
- (3) *G. hirsutum*—New World amphiploid species with $n = 26$ chromosomes.
(AD genome)

Tables 1 and 2 summarize the "marker" genes of the *G. arboreum* and *G. hirsutum* stocks respectively. Most of the symbols used were adopted from KNIGHT (1954). Genotypes of the individual *G. hirsutum* marker stocks are shown in Table 3.

SMA7 (*G. arboreum*) was crossed to *G. thurberi* and by means of colchicine treatment, a synthetic amphiploid was made. The amphiploid thus obtained which showed male sterility was crossed to each of the *hirsutum* marker stocks to make F₁ hybrids.

TABLE 1

List of marker genes used in the G. arboreum SMA7 stock

Symbol	Name
<i>cu</i>	curly leaf
<i>ne</i>	nectariless
<i>Pa, Pb</i>	complementary loci for yellow pollen
<i>r₂^{GO}</i>	ghost

TABLE 2

List of marker genes used in G. hirsutum

Symbol	Name
<i>cl</i> ₁	cluster
<i>cr</i>	crinkle
<i>cu</i>	cup leaf
<i>ne</i>	nectariless leaf
<i>p</i>	cream pollen
<i>r</i> ₂	no petal spot
<i>sb</i>	glandless
<i>v</i>	virescent yellow

TABLE 3

Genotypes of the G. hirsutum marker stocks

Stock	<i>Cl</i> ₁	<i>Cr</i>	<i>Cu</i>	<i>Ne</i>	<i>P</i>	<i>R</i> ₂	<i>Sb</i>	<i>V</i>
SM2	+	+	+	+	+	—	+	+
SM3	—	—	+	+	—	—	+	—
SM4	+	+	+	+	—	—	+	+
SM6	+	+	—	—	—	—	—	+

Method of analysis: STEPHENS (1949) formulated a theory for the analysis of amphiploids which is the basis for the method which has been used in this study. It may be summarized as follows:

Consider a particular locus, *N*, in *G. hirsutum* which has two alleles, *N* (normal) and *n* (mutant). Since *G. hirsutum* is an amphiploid species, *N* must be carried either in the A or the D subgenome. If previous studies have revealed only monofactorial segregations with respect to the *N* character in *G. hirsutum*, one of two alternatives may follow: (a) either one of the subgenomes lacks an *N* locus, or (b) if a duplicate *N* locus is present, it must be represented by an inactive allele, presumably a duplicate recessive, *n'*. Since the absence of a locus is not readily distinguishable from the presence of a duplicate recessive, both possibilities may be symbolized conveniently as "0" (zero). On this basis the following genotypes are possible:

N carried in the A subgenome: *N*_a 0_d—Normal

*n*_a 0_d—Mutant

N carried in the D subgenome: 0_a *N*_d—Normal

0_a *n*_d—Mutant

Next it is desired to find out the genetic constitutions with respect to the *N* locus of the constituent diploid species, *G. arboreum* (A genome) and *G. thurberi* (D genome). A synthetic amphiploid derived from a hybrid between these two species is crossed to normal (*N*) and recessive (*n*) stocks of *hirsutum* in order to analyze their genotypes.

With respect to the *N* character the synthetic may have the following possible genetic constitutions: (1) Both *arboreum* and *thurberi* carry *N* alleles. (2) Either

arboreum or *thurberi* carries an *N* allele, the *N* locus missing in the other species, and (3) Neither *arboreum* nor *thurberi* carries the *N* locus.

To test these three possibilities, two types of testcrosses are involved:

Type A: (Synthetic $\times$ Normal) $\times$ Mutant

Type B: (Synthetic $\times$ Mutant) $\times$ Mutant

The system of analysis presented in Table 4 applies to characters for which both diploid species are phenotypically normal. It can be extended to cases where there exists in the diploid species a mutant form which is apparently homologous with, or a duplicate of, the mutant existing in the tester *hirsutum* stock. All that is involved in such cases is the substitution of a mutant, *n*, for "*0*" in the genotype of the synthetic. Tests 2, 3, and 4 can be applied with the same expectations.

The above system of analysis is oversimplified and in practice the technical difficulties of scoring segregations from interspecific hybrids often leads to inconclusive results. Previous experience of segregating progenies in interspecific hybrids of *Gossypium* has shown that Mendelian expectations often are not realized. This may be due either to residual background effects, or to irregularities in gamete transmission, or to disturbances in dominance relations or to penetrance. These difficulties were augmented by the fact that large numbers of testcrosses attempted produced very low yields of seeds. However, consideration of Table 4 shows that three of the four expected classes can be identified on the basis of whether they produce segregating or nonsegregating progenies in Type A and

TABLE 4
System of analysis

Situation tested in synthetic amphiploid	Testcross			
	Type A		Type B	
	F ₁ genotype	Expected testcross segregation	F ₁ genotype*	Expected testcross segregation
(1) Both species carry <i>N</i> locus	$N_a N_d / N_a 0_d$ or $N_a N_d / 0_a N_d$	All normal	$N_a N_d / n_a 0_d$ or $N_a N_d / 0_a n_d$	3 normal: 1 mutant
(2) One diploid species carries <i>N</i> locus, homologous with active locus in <i>hirsutum</i>	$N_a 0_d / N_a 0_d$ or $0_a N_d / 0_a N_d$	All normal	$N_a 0_d / n_a 0_d$ or $0_a N_d / 0_a n_d$	1 normal: 1 mutant
(3) One diploid species carries <i>N</i> in duplicate locus to that active in <i>hirsutum</i>	$N_a 0_d / 0_a N_d$ or $0_a N_d / N_a 0_d$	3 normal: 1 mutant	$N_a 0_d / 0_a n_d$ or $0_a N_d / n_a 0_d$	1 normal: 1 mutant
(4) Neither diploid species carries an <i>N</i> locus	$0_a 0_d / N_a 0_d$ or $0_a 0_d / 0_a N_d$	1 normal: 1 mutant	$0_a 0_d / n_a 0_d$ or $0_a 0_d / 0_a n_d$	All mutant

* The F₁'s in Type B (situation 4) would have the mutant phenotype.

Type B testcrosses. This distinction can be made satisfactorily with quite small progenies. Discrimination between Class 1 and Class 2 depends on a distinction between bifactorial and monofactorial segregation ratios in the Type B testcross.

RESULTS AND INTERPRETATIONS

Table 5 summarizes the results obtained from Type A and Type B testcrosses involving seven recessive mutants of *G. hirsutum*. A more detailed consideration of the individual segregations and supplementary evidence will be presented in the following paragraphs.

Class 1. Dominant genes carried by both diploid genomes

The Glandless loci: Glandless stem is a character found in *G. hirsutum* which is known to behave as a simple recessive. A glandless mutant is unknown in Asiatic species and also in diploid American species (D genome).

Both *thurberi* and *arboresum* parents had normal glands. One may suppose, then, that each carries at least one dominant gene. Therefore the synthetic amphiploid should have at least two dominant genes which correspond to the *Sb* type found in *hirsutum*. In the latter it appears that only one of the genomes has a dominant gene. Segregation of (Synthetic $\times$ SM2) $\times$ SM6, with a progeny of 50 individuals, gave no glandless type plants as would be expected if both diploid parents carried *Sb* genes and one of them were at the same locus as the *Sb* carried by SM2. Type B segregation, (Synthetic $\times$ SM6) $\times$ SM6, should give 3 *Sb*:1 *sb*

TABLE 5

Summary of testcrosses involving seven recessive mutants of G. hirsutum and the synthetic amphiploid, G. arboresum $\times$ G. thurberi. The class number in the first column refers to the type of segregation expected on the basis of the analytical model presented in Table 4

Class	Mutant studied	Type A testcross			Type B testcross		
1	<i>sb</i>	obs: 50	<i>Sb</i> : 0	<i>sb</i>	obs: 34	<i>Sb</i> : 4	<i>sb</i> (+5)
		exp: all	<i>Sb</i>		exp: 32.25	<i>Sb</i> : 10.75	<i>sb</i>
2	<i>Pa</i>	obs: 188	<i>Pa</i> : 11	<i>pa</i>	obs: 43	<i>Pa</i> : 36	<i>pa</i>
		exp: all	<i>Pa</i>		exp: 39.5	<i>Pa</i> : 39.5	<i>pa</i>
3	<i>ne</i>	obs: 38	<i>Ne</i> : 12	<i>ne</i>	obs: 20	<i>Ne</i> : 20	<i>ne</i>
		exp: 37.5	<i>Ne</i> : 12.5	<i>ne</i>	exp: 20	<i>Ne</i> : 20	<i>ne</i>
3	<i>cu</i>	obs: 15	<i>Cu</i> : 35	<i>cu</i>	obs: 25	<i>Cu</i> : 20	<i>cu</i>
		exp: 37.5	<i>Cu</i> : 12.5	<i>cu</i>	exp: 22.5	<i>Cu</i> : 22.5	<i>cu</i>
3	<i>v</i>	obs: 41	<i>V</i> : 7	<i>v</i> (+23)*	obs: 39	<i>V</i> : 23	<i>v</i> (+3)
		exp: 53.25	<i>V</i> : 17.75	<i>v</i>	exp: 32.5	<i>V</i> : 32.5	<i>v</i>
3	<i>cl</i>	obs: 78	<i>Cl</i> : 34	<i>cl</i> (+8)	obs: 33	<i>Cl</i> : 20	<i>cl</i> (+1)
		exp: 90	<i>Cl</i> : 30	<i>cl</i>	exp: 27	<i>Cl</i> : 27	<i>cl</i>
3	<i>cr</i>	obs: 100	<i>Cr</i> : 25	<i>cr</i> (+12)	obs: 26	<i>Cr</i> : 39	<i>cr</i>
		exp: 102.75	<i>Cr</i> : 34.25	<i>cr</i>	exp: 32.5	<i>Cr</i> : 32.5	<i>cr</i>

* The numbers in parentheses refer to intermediate phenotypes which could not readily be scored either as "normal" or "mutant".

ratio if both diploid parents carry one dominant gene and one of them is homologous with *hirsutum sb*. The actual segregation was 34 *Sb*:4 *sb*:5 intermediate. The small number of *sb* segregates indicates the presence of at least two loci, but it does not rule out the possibility that more are involved. The two types of testcrosses considered together show that one of the diploid species has a dominant gene at a locus homologous with *Sb* from *G. hirsutum*, and there is at least one other active locus in the amphiploid.

Class 2. A dominant gene carried by the homologous diploid genome

The pollen color loci: From the available information on the inheritance of pollen color in *Gossypium*, it is known that *G. arboreum* SMA7 has two complementary loci, *Pa* and *Pb*. In *G. hirsutum* the presence of *Pa* in the A genome was determined (STEPHENS 1954) and the probable existence of the *Pb* gene was suggested. The *G. thurberi* pollen color is cream. In the material under study it is likely that only the *Pa* gene was segregating.

The hybrid *G. arboreum* $\times$ *G. thurberi* and the respective synthetic amphiploid showed yellow pollen. SM2 carries *Pa* gene whereas the other *hirsutum* stocks carry the alternative *pa*. The monofactorial segregations for pollen color in the Type B testcross (Table 5) is due presumably to the segregation of *Pa* in the A genome. It would appear that *G. thurberi* carried only recessive genes and that *G. hirsutum* did not carry any dominant gene in its D genome.

The appearance of cream pollen plants in the Type A testcross was unexpected because SM2 carries a dominant *Pa* gene located at the same locus as the *arboreum Pa*. If the *Pa* gene were the only one segregating, which seems to be the case, the presence of these cream pollen plants must be due to some other cause. In order to test their fertility, the "unexpected" cream pollen plants were selfed and crossed to SM3, but no seeds were obtained from either test. A microscopic examination of the pollen of these plants was made. It was found that most of the pollen grains were apparently normal, but there were also many "minute" pollen grains. This phenomenon might be expected since the material contains translocation heterozygotes and was probably the case in the other plants. Another characteristic found in the cream pollen plants was that although most of the pollen grains in a given flower were cream, a few of them were found to be yellow. This indicates that the presence of plants with cream pollen in the Type A testcross was probably due to some kind of cytogenetic disturbance rather than segregation of an unknown gene. This cytogenetic disturbance could be due to A-D genomic pairing (MENZEL and BROWN 1954) though it was not proven.

Class 3. A dominant gene carried by the duplicate diploid genome

The nectariless loci: The *arboreum* parent had no leaf nectaries, a condition which in the *arboreum* species is known to be determined by a simple recessive mutant, *ne*. Both *G. thurberi* and the synthetic amphiploid, *arboreum* $\times$ *thurberi*, had normal nectaries. Consequently there is probably at least one dominant gene in *thurberi* which covers the nectariless factor from *arboreum*. In *G. hirsutum*

nectariless (*ne*) carried by SM6 is a simple recessive of normal (*Ne*) carried by SM2 and no duplicate locus is known. The segregations from Type A and Type B testcrosses are shown in Table 5.

The amphiploid $\times$ nectariless from *hirsutum* backcrossed to *hirsutum* nectariless gives a good 1:1 ratio. These results indicate that *G. thurberi* carries only one dominant (*Ne*) gene. On the other hand, amphiploid $\times$ *Ne* backcrossed to *ne* gives a quite clear 3:1 segregation; therefore, *Ne* of *hirsutum* tester is different from *Ne* of *thurberi* and is probably located in the A genome. Nevertheless, this does not necessarily mean that *hirsutum ne* and *arboresum ne* are identical. A critical test would consist in transferring *ne* from *arboresum* into a *hirsutum* other than SM6. If the crosses between *hirsutum* nectariless and the plants with the *arboresum ne* gene yield only nectariless plants, the identity of nectariless from *arboresum* and *hirsutum* would be proved. The test has not been made because of lack of available material.

The cup leaf loci: This is another case in which the Asiatic parent carries a recessive gene, "curly." It is very similar in appearance to "cup" in *hirsutum*. *G. thurberi* has normal leaves and the mutant cup (*cu*) is not known in this species. The synthetic amphiploid, *arboresum* $\times$ *thurberi*, had normal leaves. Therefore, *thurberi* presumably carries at least one dominant gene which covers curly from *arboresum*. The cup (*cu*) gene in *hirsutum*, carried by SM6, is a simple recessive of normal (*Cu*) carried by SM2. The synthetic $\times$ SM6 had normal (*Cu*) phenotype. The Type A and Type B testcross segregations are shown in Table 5.

The monofactorial segregation obtained in the Type B test suggests that *G. thurberi* carries only one normal gene able to cover both Asiatic and New World "cup" loci. The same testcross also provided information indicating that cup and nectariless were linked. Their joint segregations obtained in the testcross and in the selfed progeny from one of its normal segregates are shown in Table 6.

Although both families were small, they give clear evidence of linkage. At the same time the large amount of intermediate-type plants for the cup character

TABLE 6

Linked segregations of cup and nectariless (a) in the testcross (Synthetic $\times$ SM6) $\times$ SM6, (b) in the selfed progeny from a normal plant (G 1-27) selected from the same testcross

	Nectararies	Nectariless	Total
(a) Noncup	17	8	25
Cup leaf	3	12	15
Total	20	20	40
	Nectararies	Nectariless	Total
(b) Noncup	8	0	8
Intermediate	36	2	38
Cup leaf	1	16	17
Total	45	18	63

indicates that the dominance of the *Cu* gene from *thurberi* is lost on the heterogeneous background produced by selfing.

In the Type A testcross, (Synthetic $\times$ SM2) $\times$ SM3, *Cu* and *Ne* genes segregate from two different loci, one coming from *thurberi*, where *Cu* and *Ne* seem to be linked, and the other from *hirsutum* SM2. STEPHENS (personal communication) studied the segregation of cup and nectariless in the progenies of two F_2 *hirsutum* families (S5563 and S5557), where cup and nectariless were in coupling phase. Out of a total progeny of 169 plants the segregation of cup and nectariless genes was as follows: 122 (*Cu-Ne*):15 (*Cu-ne*):8 (*cu-Ne*):24 (*cu-ne*). From the analysis of these data STEPHENS found that cup and nectariless loci are linked in *G. hirsutum*, with 15 percent crossing-over.

The nectariless data indicated that *Ne* from *thurberi* is not homologous with *Ne* of the SM2 *hirsutum* stock. Therefore the linkage *Cu-Ne* of *hirsutum* should be different from the one which seems to be present in *G. thurberi*. If the assumption is made that two *Cu* genes are necessary to have noncup phenotype, the expected segregation ratio in Type A testcross (omitting crossover types) would be: 1 noncup with nectaries:2 cup with nectaries:1 cup, nectariless. The actual segregation of cup and nectariless loci from the testcross (Synthetic $\times$ SM2) $\times$ SM6 is given in Table 7.

These data agree with the expected ratio. The two plants which had the phenotype, noncup-nectariless, were probably crossover types. The data as a whole indicate the existence of a linkage group *Cu-Ne* in *thurberi* which is different from the linkage *Cu-Ne* found in *hirsutum*. The *Cu-Ne* group is presumably located in the A genome of the latter species. The most likely explanation is that these *hirsutum* genes are homologous with *cu* and *ne* genes of *G. arboreum*. Since the association between curly and nectariless in *arboreum* is not known, the latter interpretation cannot be proven.

The virescent yellow loci: Virescent yellow is a mutant known to behave as a single recessive gene in *G. hirsutum*. Both diploid parents and the corresponding synthetic amphiploid had normal phenotypes. The F_1 obtained by crossing the synthetic amphiploid with SM3 (*hirsutum* stock carrying virescent yellow) was normal, which indicates the presence of at least one dominant (*V*) gene in the synthetic parent.

The Type A and Type B testcrosses are included in Table 5. The Type B cross shows that only one normal gene from the amphiploid is involved, since the segregation approaches a 1:1 ratio. The Type A testcross segregated virescent yellow plants, which shows that the normal gene in the synthetic amphiploid is

TABLE 7

Segregation of cup and nectariless genes from the testcross (Synthetic $\times$ SM2) $\times$ SM6

	Nectaries	Nectariless	Total
Noncup	13	2	15
Cup leaf	25	10	35
Total	38	12	50

at a different locus from *V* of *hirsutum*. In spite of the poor fit to the expected 3:1 ratio in this case, it is unlikely that more than two genes are involved, because there is only one gene difference between the *hirsutum* stocks SM2 and SM3 with respect to the virescent character.

Assuming that each genome carries only one gene for virescent, the *hirsutum* allele must be in a different genome from the dominant gene in the synthetic amphiploid. The location of virescent in *hirsutum* is not known nor is its linkage relationship with other genes determined. Thus the only way the above hypothesis can be tested at present is by crossing virescent *hirsutum* with each of the diploid parents. *Hirsutum* $\times$ *arboreum* is quite difficult to obtain. Therefore, to avoid this impasse a tetraploid *arboreum* was crossed to SM3. Two plants were obtained. These two plants showed intermediate phenotype for virescent, indicating that *arboreum* does not properly cover the recessive character in study. Dosage effect is probably the cause for the intermediate virescent appearance of these two plants. The most likely explanation is that *arboreum* does not carry any gene dominant to *hirsutum v* and that probably virescent in *hirsutum* is located in the A genome or in a D locus different from that in *thurberi*. To complete the investigation it would be necessary to cross SM3 $\times$ *thurberi*. GERSTEL (personal communication) succeeded in getting out of this cross one plant which had green leaves, suggesting the presence in *thurberi* of a dominant gene for virescent, and that the virescent locus in *hirsutum* is probably located in the A genome.

The cluster loci: Cluster is a character which in *hirsutum* is known to be determined by a simple recessive mutant located in the D subgenome. Both parental diploid species, *G. arboreum* and *G. thurberi* had normal branching habits. The synthetic amphiploid and the F_1 obtained by crossing this synthetic with *hirsutum* cluster had the normal phenotype also, indicating that there is at least one dominant allele in the synthetic which covers cluster from *hirsutum*. Type A and Type B testcross data are given in Table 5.

The fact that both *arboreum* and *thurberi* had normal phenotypes for this particular character suggests that each is carrying one or more *Cl* genes; nevertheless, the family from the Type A testcross segregated for cluster. This could happen only if *thurberi* does not have a dominant allele homologous with cluster from *hirsutum*. At the same time, the Type B segregation indicates that only one dominant (*Cl*) comes from the amphiploid. Type A segregation indicates that this *Cl* from the amphiploid is at a different locus from the Cl_1 of *hirsutum*.

One way of identifying the genome location of the *Cl* locus in the synthetic amphiploid is by using the tool of duplicate *R-Cl* linkage groups, which are found in the A and D subgenomes of the New World cultivated species (HARLAND 1937; SILOW 1946), and loci in A and D diploid species which are homologous with those of the New World species. If the petal spot gene (r_2^{go}) of *arboreum* is found to be linked with the *Cl* found in the amphiploid, this would indicate that the dominant *Cl* is in the A genome of the amphiploid.

The parental constitution with respect to petal spot is as follows: *G. arboreum* carries a ghost spot (r_2^{go}) gene; *G. thurberi* carries a weak red petal spot, which

is not recovered in the first backcross and successive generations; and all the *hirsutum* parental stocks carry a spotless gene (r_2) located in the A subgenome. The hybrid obtained on crossing spotless *hirsutum* with the ghost type of *arborescens* produces a red spot (SILOW 1941).

The study of the expected association between petal spot and cluster was made in the Type B testcross where the only dominant *Cl* comes from the synthetic amphiploid. The segregations and the χ^2 test for independence are in Table 8.

A large deficiency of plants with red spot was observed, which was probably due to the instability of the petal spot gene. This phenomenon was noted earlier by other workers. The fact that among the red spot plants the ones with non-cluster type were three times as many as the cluster ones suggests an association between petal spot and *Cl*. However, the individual chi-square tests showed that there was no significant interaction between petal spot and cluster, and that the deviation from equality shown in the total chi-square was due mainly to the abnormal segregation of petal spot.

The segregations in Type A and Type B testcrosses indicate that *thurberi* does not carry a dominant *Cl* homologous with *hirsutum* cluster. According to this interpretation cluster segregates should be of two types, one which would breed true to cluster ($0_a cl_d/0_a cl_d$), and the other ($0_a 0_d/0_a cl_d$) should segregate types which contained neither *Cl* nor *cl* genes (normal phenotypes?). Two cluster plants were selfed and gave the following results:

Family	<i>Cl</i>	<i>cl</i>	Total
G4-37 selfed	0	29	29
G4-45 selfed	4	37	41

These results agreed with the interpretation. Nevertheless, the presence of the four noncluster plants in family G4-45 (selfed) might be attributed to the presence of modifiers. A critical test would consist of selfing these four plants to see whether they breed true. This test could not be made during the available time.

Supporting evidence of the hypothesis that *arborescens* carries a normal *Cl* allele was shown by the normality of two hybrid plants, tetraploid *arborescens* $\times$ SM3. However, this test is not critical because of possible dosage effects. The absence

TABLE 8

Segregation of cluster and petal spot genes from the testcross (Synthetic $\times$ SM3) $\times$ SM3

	Red spot	Spotless	Total
Noncluster	13	19	32
Cluster	4	16	20
Total	17	35	52

χ^2 test for independence			
<i>Cl</i> vs. <i>cl</i>	: $\chi^2(1) = 2.77$	$P(1) = 0.10$	-0.05
<i>R</i> vs. <i>r</i>	: $\chi^2(1) = 6.23$	$P(1) = 0.025$	-0.01
Linkage	: $\chi^2(1) = 0.69$	$P(1) = 0.80$	-0.70
Total	: $\chi^2(3) = 9.69$	$P(3) = 0.025$	-0.01

of a *Cl* gene from *thurberi* could be confirmed by crossing SM3 with *thurberi*, but this combination has not yet been made.

The study of association between petal spot and cluster, in the A genome, was made also in the Type A testcross. In this testcross, two *Cl* genes are segregating, one of them coming from the SM2 stock and the other from the synthetic amphiploid. Since the cluster locus in *hirsutum* is located in the D genome, and the segregating locus for petal spot is situated in the A genome, the test consists in finding out whether the dominant *Cl* from the synthetic is associated with petal spot. If it is assumed that both *Cl* loci are independent from petal spot, the following ratio would be expected: 3(*Cl-R*):3 (*Cl-r*):1 (*cl-R*):1 (*cl-r*). On the assumption of complete linkage association between the dominant *Cl* of the synthetic amphiploid and the *arboreum* petal spot gene, the expected segregation ratio would be 2 (*Cl-R*):1 (*Cl-r*):0 (*cl-R*):1 (*cl-r*).

The actual data are given in Table 9. There is a fairly regular segregation for petal spot as well as for cluster, and the linkage between the dominant *Cl* and petal spot in the A genome becomes quite clear. This evidence along with that presented previously indicates the existence of duplicate cluster loci, one of which (*hirsutum* cluster) is known to be located in the D genome, and the other possibly coming from *G. arboreum* (A genome).

The crinkle loci: In *G. hirsutum* crinkle is a condition which is known to be determined by a simple recessive mutant (*cr*). It is also known that crinkle is located in the D subgenome of the cultivated New World species. In the two parental diploid species the presence of crinkle has not been reported. The synthetic amphiploid made from the hybrid *arboreum* × *thurberi* and the F₁ obtained by crossing this synthetic with *hirsutum* crinkle (SM3) had normal phenotype, indicating the presence of at least one dominant (*Cr*) allele in the synthetic which covers crinkle from *hirsutum*.

The Type B testcross (Table 5), because of its monofactorial segregation, indicates that there is only one dominant (*Cr*) gene in the amphiploid which covers the *hirsutum* crinkle. Segregation in testcross A indicates that this (*Cr*) gene is not homologous with *Cr* of SM2. The data in Table 5 could be explained, then, by saying that either *G. arboreum* carries a dominant allele which covers the

TABLE 9

Segregation of cluster and petal spot genes from the testcross (Synthetic × SM2) × SM3

	Red spot	Spotless	Total
Noncluster	40	34	74
Cluster	7	27	34
Total	47	61	108

χ^2 test for independence			
<i>Cl</i> vs. <i>cl</i>	: $\chi^2(1) = 2.40$	$P(1) = 0.20-0.10$	
<i>R</i> vs. <i>r</i>	: $\chi^2(1) = 1.81$	$P(1) = 0.20-0.10$	
Linkage	: $\chi^2(1) = 13.44$	$P(1) = \text{less than } 0.005$	
Total	: $\chi^2(3) = 17.65$	$P(3) = 0.01-0.005$	

hirsutum crinkle, or the dominant allele in the synthetic is located in *thurberi* but is in a chromosome nonhomologous to the *hirsutum* chromosome with the crinkle character. The hybrid tetraploid *arboreum* $\times$ SM3 had normal phenotype suggesting the presence of a dominant (*Cr*) character in *arboreum*. However, this is not critical because of possible dosage effects.

According to the interpretation that *G. thurberi* does not carry a dominant (*Cr*) gene homologous with *Cr* of *hirsutum*, crinkle segregates from testcrosses A or B should be of two types, one of which would breed true ($0_a cr_d/0_a cr_d$) and the other ($0_a 0_d/0_a cr_d$) should segregate types which contain neither *Cr* nor *cr* ($0_a 0_d/0_a 0_d$) when selfed. These plants should show normal phenotype if viable. The plant G4-37, which had crinkle phenotype, was selfed giving a progeny composed of five normal plants and 28 crinkles. This result agrees with the interpretation presented above. The possible presence of modifiers, mentioned in the case of cluster, needs to be tested.

DISCUSSION

Testcrosses of the hybrid, synthetic amphiploid (*G. arboreum* $\times$ *G. thurberi*) $\times$ *G. hirsutum* marker stocks were used to test homologies between the chromosomes of the two diploid species and the corresponding A and D subgenomes of *G. hirsutum*.

The eight marker genes of *G. hirsutum* used for the present analysis were characterized by their monofactorial segregations. Their location in the corresponding *hirsutum* subgenome has already been found for four of these mutants (STEPHENS 1954, 1955). At present, the probable location of the cup leaf, nectariless and virescent yellow loci is indicated by the results obtained, whereas the genome location of the glandless mutant remains still unknown.

It is important to note that although *G. hirsutum* is a tetraploid, it behaves cytologically as a functional diploid; and that from the more than 30 mutants known only six are duplicate, whereas the others have monofactorial segregations. The latter implies that *hirsutum* behaves also as a diploid genetically.

The material provided by *G. hirsutum*, which is a natural allotetraploid, and the putative diploid parents in existence, are suitable for the study of species differentiation in the genus *Gossypium*. In this respect it would be interesting to learn why most of the mutants of *G. hirsutum* segregate monofactorially, knowing that this species was originated from two distantly related diploid species. Two possibilities may be pointed out to explain this phenomenon:

(1) Both diploid parents had many loci in common; consequently the original amphiploid would have many duplicate loci. In this case by a process of diploidization one of each of duplicate loci has become genetically inactive. This could happen if one of the duplicate loci had become homozygous for a recessive mutant or had lost its ability to cover mutants at the other locus.

(2) The diploid parents were sufficiently differentiated to the point that they might carry few loci in common. In this case the original amphiploid would not have duplicate loci for these genes.

According to the first possibility, one would expect that the related species for both genomes (A and D) would tend to carry dominant genes capable of covering the *hirsutum* mutant. On the other hand, in the second situation it would be expected that only the homologous species would carry a dominant covering a *hirsutum* mutant in the A subgenome, and the D species carry dominants covering mutants in the D subgenome.

The evidence given in the results section does not support the second possibility. In the cases of cup leaf, nectariless and glandless, it was found that both diploid species, *G. arboreum* and *G. thurberi*, carried dominant genes which were able to cover these *hirsutum* mutants. As a matter of fact, cup and nectariless genes, which are linked in *hirsutum* and presumably located in the A subgenome, were covered by dominants from *thurberi* (D genome), in which these genes were also found to be linked. There were also other situations in which a cross-masking effect was observed. In other words, *G. arboreum* carries a dominant which covers a mutant in the D subgenome, as in the case of cluster and crinkle, or a mutant in the A subgenome is covered by a dominant from *thurberi*, which is probably the case in virescent yellow. These explanations would favor the first possibility of species differentiation mentioned above; however, there were cases which cannot properly be interpreted by this model. One of them is concerned with the *thurberi* petal spot, which does not cover spotless situated in the duplicate genome. Another is the noncluster from *thurberi* which does not cover the cluster located in the homologous genome, as is also the case for crinkle. A final example is the nonvirescent from *arboreum* which does not cover virescent yellow in the homologous genome.

The logical explanation of these results would be that the nonmutant gene from the diploid species lacked dominance over the mutant when transferred to the *hirsutum* background. Therefore, instead of masking the mutant gene, they would behave as recessives, which could be extracted on selfing the mutant phenotype. This may explain why the plant G4-45, which had cluster phenotype, when selfed yielded some normal plants and that the crinkle plant G4-37 also segregated normal plants after being selfed. However, if this is the correct interpretation, it is difficult to see why the duplicate gene from the opposite species retains its dominance on the same background. If the genes from one of the diploid species lose their dominance on the *hirsutum* background, it would be expected that the other would do so in the same way. On the other hand, if the expression of dominance is due to the presence of modifiers, it would imply that duplicate genes show different and specific modifier requirements. What is more, it would require that genes in duplicate loci are less affected by modifiers than genes at the same locus. In this respect, FISHER's theory of the evolution of dominance (1928) would fit the cluster case because in Asiatic diploids a cluster mutant is known (PATEL, MUNSHI and PATEL 1947). Thus dominance would be built up in *arboreum* which might cover the *hirsutum* cluster located in the A subgenome, as well as the cluster character of species in the A subgenome. However, FISHER's theory could not explain the case of crinkle since this mutant is unknown in Asiatics.

In the cases of cluster and crinkle loci, both of which are located in the D subgenome of *hirsutum*, it was observed that *G. thurberi* was the species lacking dominant alleles. It is entirely possible that *thurberi* is rather distantly related to the original (D genome) diploid parent and is sufficiently differentiated from the true parental species so that some of the genes are not held in common. In fact, cytological evidence has shown that *thurberi* is not closely related to the original D ancestor (GERSTEL and PHILLIPS 1958). The same authors have shown that *G. raimondii* (another D species) is cytologically closer related to the D subgenome of *hirsutum* than *thurberi* is. They found also that cluster and crinkle from *hirsutum* are covered by *raimondii* genes in hexaploid hybrids between these two species. It is interesting to notice that *G. raimondii*, which is cytologically closely related to *hirsutum* (D subgenome), carries dominant genes for cluster and crinkle; whereas *thurberi*, which is not closely related to *hirsutum*, apparently lacks dominant genes for these mutants. However, there is still the possibility that none of the existing diploid species are the same as the original A and D diploids which were the *G. hirsutum* parents. Although species within the same genome are related closely enough to produce fertile hybrids, there is the likelihood that small structural changes have occurred over the years. This might be especially the case in species that formed amphiploids, which had become adapted to their new genetic structure.

SUMMARY

A synthetic amphiploid obtained from the hybrid, *G. arboreum* $\times$ *G. thurberi* was crossed to multiple dominant and recessive *G. hirsutum* marker stocks and testcrossed to *hirsutum* in order to make a comparative genetic analysis of the two diploid species and the corresponding A and D subgenomes of *G. hirsutum*.

The method of analysis was based on the one formulated by STEPHENS (1949).

The probable location of cup leaf, nectariless and virescent yellow mutant genes in the A subgenome of *G. hirsutum* was postulated.

The presence of dominant normal genes in the two diploid species with respect to the *hirsutum* mutants in test was analyzed. The testcross data indicated that in the cases of cluster, crinkle and virescent yellow the related diploid species did not carry a dominant gene homologous with the *hirsutum* tester, in spite of the "normal" phenotype of this species with respect to the *hirsutum* recessive mutant. In these cases, however, it was observed that duplicate genes from the opposite species had a cross-masking effect over the *hirsutum* mutants. Some possible interpretations are discussed.

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