A CYTOLOGICAL METHOD FOR GENOME ANALYSIS IN GOSSYPIUM

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IN AN earlier paper (MENZEL and BROWN 1952), the behavior of a radiation-induced reciprocal chromosome translocation in *Gossypium hirsutum* L. was described. This translocation (designated 2B-1) had two salient features which promised to be of considerable convenience for further cytogenetic analyses. (1) The interchanged arms were very unequal, almost the whole arm of one chromosome ("chromosome 2") having exchanged places with a very short segment of another chromsome ("chromosome 1"). As a consequence, at metaphase I in the translocation heterozygote, an interstitial chiasma was present proximal to the point of attachment of the longer ("A") arm in about 98% of the pollen mother cells, whereas the shorter translocated ("x") end was never paired. These relationships made it possible to determine the presence or absence of chiasmata at metaphase I with a high degree of accuracy at six different positions in the IV. (2) Because of these morphological features, it was possible to demonstrate that a wide array of viable, fertile deficiency-duplication genotypes was recovered from the translocation heterozygote.

The present paper reports the use of the 2B-1 translocation in further studies designed to determine the genome affinities of the two chromosomes involved and to analyze the behavior of corresponding chromosomes introduced into *G. hirsutum* from its diploid relatives.

Cytological analyses were made from temporary iron-acetocarmine smears of pollen mother cells. To facilitate discussion and illustration, numerals or letters have been assigned to chromosomes and chromosome ends and segments involved in the studies reported here. It should be emphasized that the system adopted here is provisional, and not intended as a permanent scheme for the genus. The latter must await the accumulation of additional stocks and more complete data.

GENOME AFFINITIES OF CHROMOSOMES 1 AND 2 OF THE 2B-1 TRANSLOCATION

It has been amply demonstrated that the amphidiploid species of Gossypium, including *G. hirsutum*, have one set (or subgenome) of 13 chromosomes similar to those of the wild American diploid species of Gossypium, while the other subgenome of 13 chromosomes is similar to those of the diploid Asiatic cultivated species. Following BEASLEY (1940), the genome symbol $(AD)_1$ designates the haploid chromosome set of *G. hirsutum*, while A and D refer, respectively, to the Asiatic cultivated and the American wild species. The two diploid species employed in the present study were *G. raimondii*, D_5, and *G. herbaceum*, A_1. Subgenomes of *G. hirsutum* are referred to separately as A_h and D_h (BROWN and MENZEL 1952b).

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When *G. hirsutum* is crossed with a D-genome species, the F_1 hybrid characteristically forms 13 small D II's and 13 A_h I's at metaphase I, with a low frequency of III's, averaging less than one per cell, due principally to pairing of A_h chromosomes with D II's (for review, see BROWN and MENZEL 1952a). Similarly, when *G. hirsutum* is crossed with an A-genome species, the A and A_h chromosomes pair, and the D_h chromosomes remain largely unpaired (reviewed by GERSTEL 1953).

It is possible, therefore, to assign *hirsutum* chromosomes which are marked by translocations to their respective subgenomes by crossing a plant carrying the trans-

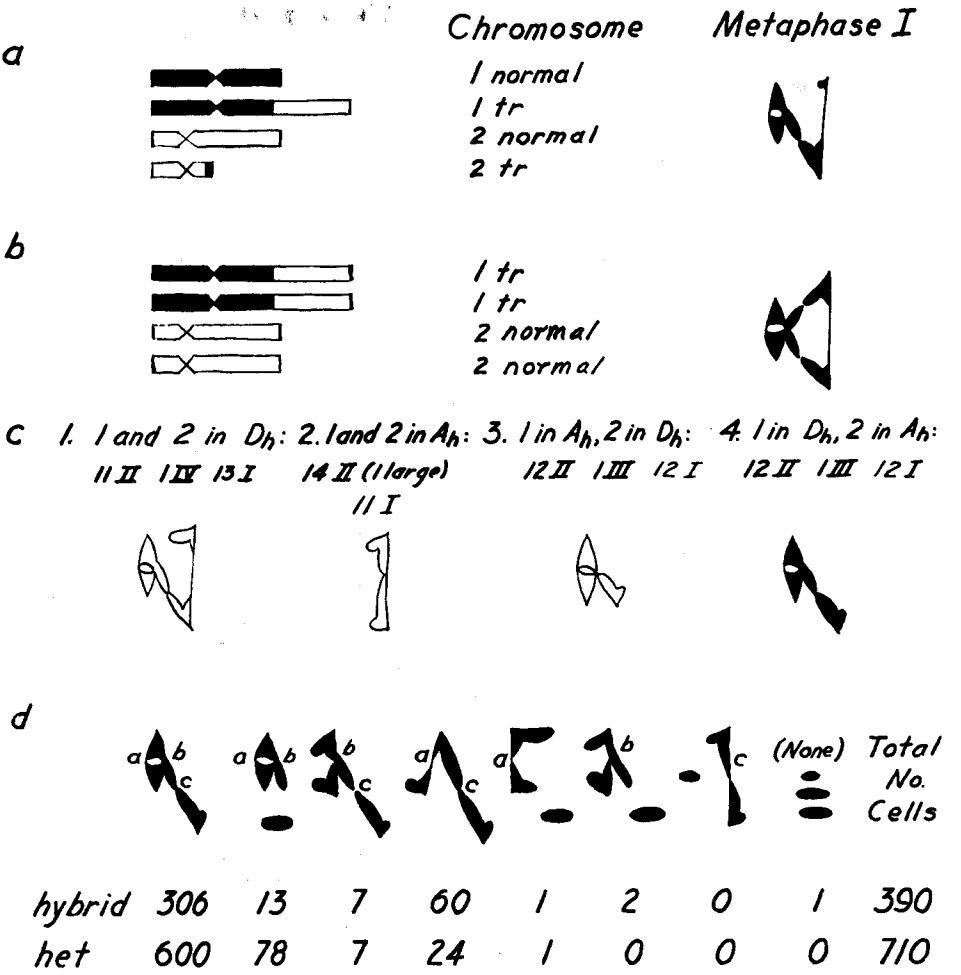
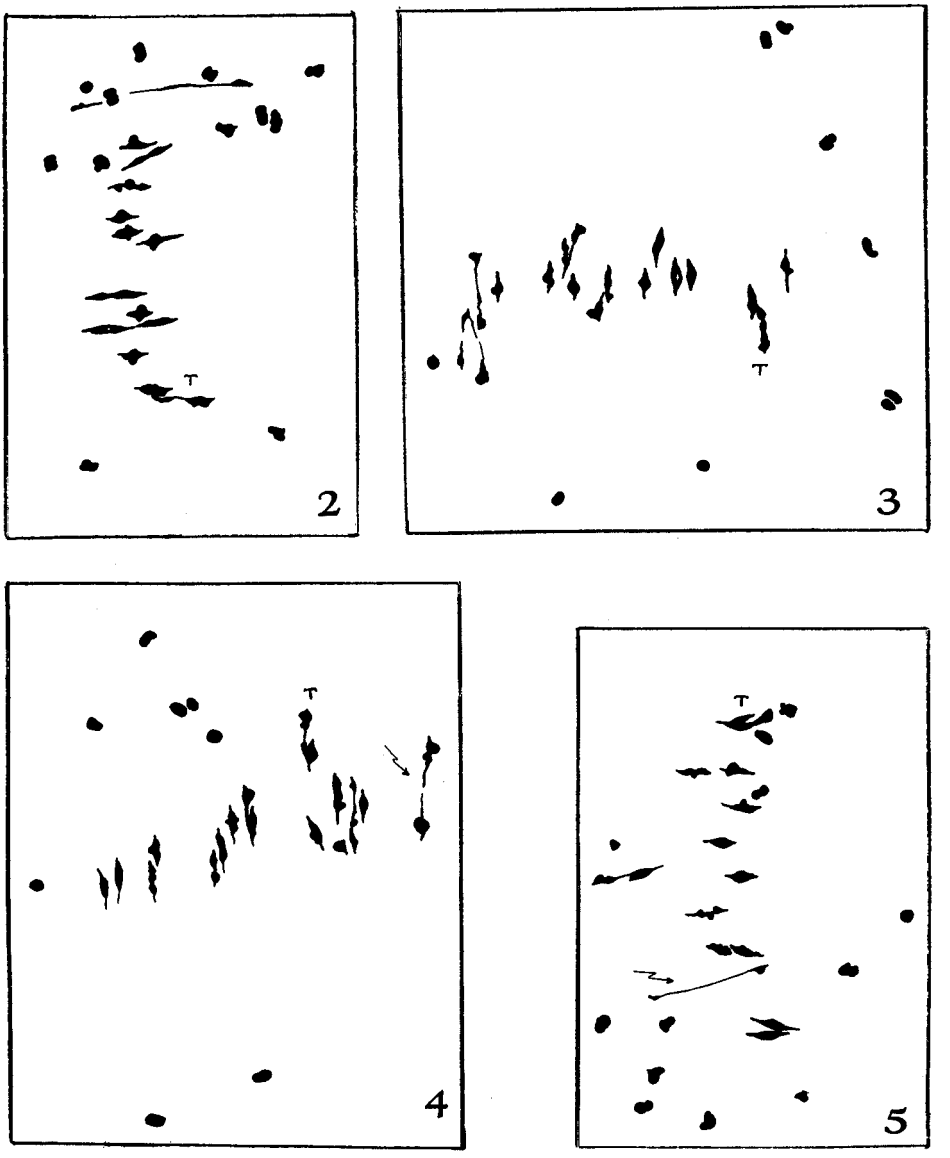


FIGURE 1. a. Chromosome structure and MI configuration of the heterozygous 2B-1 translocation. b. Chromosome structure and MI configuration of the 2n + 2A − 2x deficiency-duplication dwarf. c. Identifying configurations expected in the F_1 hybrid 2n + 2A − 2x × *G. raimondii* for the four possible genome affinities of chromosomes 1 and 2 of the 2B-1 translocation. Contingency number 4 proved to be the correct one. d. Frequency of translocation III's observed in the triploid hybrid and in the translocation heterozygote with various combinations of chiasmata at positions a, b, and c. In the case of the heterozygote, the fourth possible chiasma (see fig. 1a) is ignored.



FIGURES 2–5. Metaphase I configurations in pollen mother cells of the triploid hybrid 2n + 2A − 2x × *G. raimondii*. The III which is due to the translocation is marked "T" in each figure. The other III's are due to intergenomic ($D_hD_5A_h$) association.

FIGURE 2. A typical cell with 12 II 1 III 12 I.

FIGURE 3. The maximum association seen: 9 II 4 III 9 I.

FIGURE 4. 12 II 2 III 9 I. The arrow indicates a loose II composed of two A_h chromosomes.

FIGURE 5. 10 II 2 III 13 I. The arrow indicates a dividing, or misdividing, I. One of the potential D_5D_h II's has failed to form and is represented by two I's smaller than the rest.

located chromosomes to either an A or a D species, or to both, and comparing the pairing in the F_1 hybrids with that in hybrids carrying the normal *hirsutum* arrangement.

In practice, however, the analysis is limited to (AD)D hybrids for two reasons. (1) Hybrids between *G. hirsutum* and the two A-genome species, *G. arboreum* and *G. herbaceum*, are very difficult to obtain, while *G. hirsutum* crosses readily with several of the D-genome species. (2) It has been shown (GERSTEL 1953, MENZEL and BROWN 1954) that A_1, A_2 (*arboreum*) and A_h differ in the end arrangement of some of their chromosomes, so that $(AD)_1A_1$ F_1 hybrids form 9 II 2 IV, and $(AD)_1A_2$ F_1 hybrids form 8 II 1 IV 1 VI, instead of 13 II. Hence analysis of newly rearranged chromosomes in *hirsutum* by means of such hybrids would be complicated by the associations of IV and VI already present.

To determine the genome affinities of the chromosomes involved in the 2B-1 translocation, a cross was made between a derived deficiency-duplication dwarf of the genotype 2n + 2A − 2x (MENZEL and BROWN 1952) and *G. raimondii*. Figure 1 shows diagrammatically the chromosome structure of the 2B-1 heterozygote (figure 1a) and of the dwarf parent used in the cross (figure 1b), and the identifying configurations expected in the F_1 hybrids for the four possible genome affinities (figure 1c), namely if (1) both chromosome 1 and chromosome 2 were in the D_h subgenome, (2) both chromosomes were in the A_h subgenome, (3) chromosome 1 were in the A_h and chromosome 2 in the D_h subgenome, or (4) chromosome 1 were in the D_h and chromosome 2 in the A_h subgenome.

Pairing in the F_1 hybrids, illustrated in figures 2–5, conformed with the fourth contingency; that is, chromosome 1 is in the D_h subgenome and chromosome 2 is in the A_h subgenome. Pairing was analyzed in 390 cells of the hybrids. A total of 436 III's were seen, an average of 1.12 per cell. Of these, 63 (0.16 per cell) were due to DDA III's and 373 were due to the translocation. No difficulty was encountered in distinguishing the two types of trivalents by means of the long translocated A arm in the latter. The two types of trivalents are illustrated in figures 2–5. The two chromosomes 1 were paired in one or both arms in all but one cell. In a single cell a loosely-paired II composed of two A_h chromosomes was seen (fig. 4). In another cell (fig. 5), division or misdivision of an A_h I appeared to be in progress.

COMPARISON OF CHROMOSOME 1 OF D_5 AND CHROMOSOME 1 OF D_h

The ease with which the translocation III could be recognized in the triploid hybrids made it possible to compare the frequencies of chiasmata at three positions (designated a, b, and c, fig. 1d) in the III at metaphase I in the triploid, where the normal chromosome 1 was from D_5 and the altered chromosome 1 from D_h, with the frequencies of chiasmata at the same positions in the translocation heterozygote, where both normal and altered chromosome 1 were from D_h. Figure 1d indicates the frequency of configurations due to various combinations of the three chiasmata in the two types of plants.

Table 1 shows the frequencies of each of the three chiasmata in the hybrid and in the translocation heterozygote. A chi-square analysis of these data was carried

TABLE 1

Frequency of chiasmata at positions a, b, and c in (I) the 2B-1 heterozygote and (II) the 2n + 2A − 2x × D_5 triploid hybrid

Date of sample	Frequency of chiasmata at						Total PMC
	a		b		c		
	No.	%	No.	%	No.	%	
I							
Summer 1951	139	*100.0*	137	*98.6*	118	*84.9*	139
Summer 1952	145	*97.3*	138	*92.6*	122	*81.9*	149
Summer 1953	294	*99.7*	286	*96.9*	269	*91.2*	295
Winter 1953–54	127	*100.0*	122	*96.1*	122	*96.1*	127
Total	705	*99.3*	683	*96.2*	631	*88.9*	710
II							
10–1–53	102	*99.0*	87	*84.5*	98	*95.1*	103
11–13–53	196	*98.0*	168	*84.0*	193	*96.5*	200
11–13–53	30	*93.7*	28	*87.5*	30	*93.7*	32
11–16–53	52	*94.5*	44	*80.0*	51	*92.7*	55
Total	380	*97.4*	327	*83.8*	372	*95.4*	390

	χ^2 (1 d.f.)	P
aI vs. aII	4.11	<0.05
adjusted	3.15	>0.05
bI vs. bII	52.91	<0.01
cI vs. cII	14.49	<0.01

out by DR. M. J. GARBER of the Department of Genetics, A. and M. College of Texas.

The frequency of chiasmata at position a, in the unaltered arm of chromosome 1, does not differ greatly between the *raimondii* hybrid and the heterozygote. It was present in 97.4% of PMC's in the former and 99.3% in the latter. The adjusted chi-square of 3.15 is not significant.

The frequency of the interstitial chiasma proximal to the point of interchange, position b, on the other hand, was consistently *lower* in the triploid (83.8% of PMC's in the triploid, 96.2% in the heterozygote). The chi-square value of 52.91 is highly significant.

It appears, therefore, that when a D_5 chromosome 1 is substituted for the normal D_h chromosome in the configuration, chiasmata are reduced significantly in the "right" arm but not in the "left" arm.

Comparison was also made of the chiasma frequency at position c, between the translocated and the normal arm of chromosome 2. Since these segments were from A_h in both cases, no difference in chiasma frequency was expected. However, the frequency was found to be appreciably *higher* in the triploid (95.4%) than in the heterozygote (88.9%). The difference is statistically significant.

It will be noted that chiasmata are reduced at b and increased at c in the hybrid as compared with the heterozygote. This suggests that there is some interference or competition between b and c in the translocation heterozygote. When this interference is reduced as a consequence of the lower chiasma frequency proximal to the point of interchange in the right arm when a D_5 and a D_h chromosome 1 are present together, chiasma formation at c rises accordingly.

COMPARISON OF CHROMOSOME 2 OF A_h WITH CHROMOSOME 2 OF A_1

Because of the crossing difficulties mentioned above, it was not feasible to compare chromosome 2 of 2B-1 directly with its A_1 homologue in a triploid F_1 hybrid. However, GERSTEL (1953) has shown that the *hirsutum-herbaceum* F_1 hybird characteristically forms 2 IV at metaphase I, due to different end arrangements of four chromosomes in A_1 and A_h. The A_1 translocation complexes may be introduced into fertile, 52-chromosome plants by first doubling the F_1 hybrid with colchicine, and then backcrossing to *G. hirsutum* until aneuploidy has been eliminated (MENZEL and BROWN 1954). Some of the resulting 52-chromosome plants have one or both of the IV complexes. One such plant (Z850), which had metaphase pairing of 24 II 1 ring IV, was crossed with a plant homozygous for the 2B-1 end arrangement. Cytological analysis of ten F_1 plants gave the following results:

(1) Five plants had only the heterozygous 2B-1 configurations and received only normal A_h chromosomes from Z850.

(2) Two plants formed a figure of VI in most cells, proving that chromosome 2 of 2B-1 is one of the chromosomes involved in the IV of Z850. In other words, chromosome 2 is one of the 4 A_h chromosomes which differ in end arrangement from the chromosomes of A_1. These two plants received two chromosomes with the A_1 end arrangement from Z850.

(3) Three plants showed configurations different from either of the above types. They conformed with the pairing expected if two complementary deficiency-duplications were functioning from the IV complex in Z850.

The maximum configurations expected if the two balanced and four numerically equal unbalanced gametes from Z850 function are shown in figure 6. The arms of the A-genome differential chromosomes have been assigned numbers from 1 to 10 (MENZEL and BROWN 1954). At present we do not know which of eight chromosome arms (arm 3 to arm 10) was involved in the 2B-1 translocation.[2] Figure 6 has been drawn on the assumption that chromosome 2 is chromosome 3–5 of A_h and that arm 5 is the translocated or A arm. The configurations would be the same regardless of which of the eight arms is actually involved. The D_h chromosome 1 is designated 27–28 for convenience, with end 28 corresponding to the short, non-pairing translocated or x segment. Two of the deficiency-duplications recovered corresponded to type 5 of figure 6 and one to type 3.

Insufficient data have been obtained to allow a comparison of chiasma frequencies when an A_1 chromosome is substituted for the A_h chromosome 2 in the configuration.

[2] Since this paper was prepared, further studies have shown that chromosome 2 of the 2B-1 translocation must be either chromosome 3-5 or chromosome 4-6 of A_h.

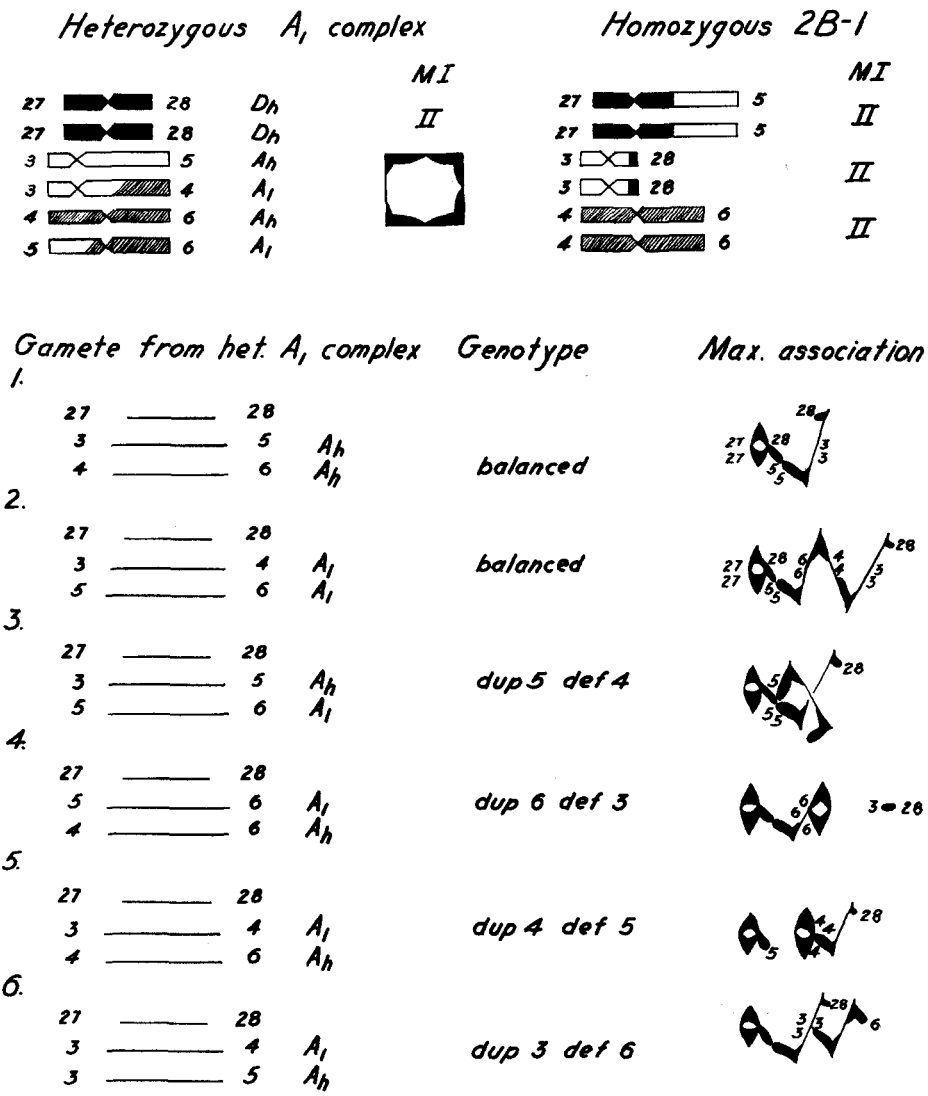


FIGURE 6. The chromosome end arrangements of Z850, which is heterozygous for two of the A_1 differential chromosomes; the end arrangements of plants homozygous for the 2B-1 translocation; the six genotypes expected if all the balanced gametes and the four numerically equal nondisjunctional gametes all function in a cross between the two; and the maximum metaphase association expected for each of the six genotypes. This diagram is based on the assumption that chromosome 2 of the 2B-1 translocation is differential chromosome 3-5 of A_h, and that end number 5 was the translocated end. The normal end arrangement of *G. hirsutum* in this scheme is 3-5, 4-6, 27-28.

However, even if such a comparison were made it would not be comparable to the study of chromosome 1, since after several generations of backcrossing a large portion of the content of the original A_1 arm may have been replaced by A_h segments, even though the A_1 end arrangement has been retained.

DISCUSSION

The differences in end arrangement existing in nature among the A_1, A_2 and A_h genomes are of particular interest because of the meiotic complexities they produce in three-species tetraploid hybrids of the genome constitution (AD)AD. Several such hybrids are being used as a source of primary breeding stocks at this station. Inconclusive data from this material (MENZEL and BROWN 1954, BRADFORD 1954, A. M. MEMON unpublished) had suggested that deficiency-duplications are recovered from the A-genome multivalent complexes. But analysis in the three-species hybrid material was impaired by the lack of morphologically marked chromosomes and by the occurrence in such material of an unexpectedly high frequency of excess multivalents attributed to intergenomic (AD) pairing. The present study demonstrates conclusively that both duplications and deficiencies for at least two ends of the five differential chromosomes are functional. It should eventually be possible, with other suitable translocation lines, to analyze in considerable detail both the dosage effects and the genetic contents of the differential A-genome chromosomes.

Substitution of a D_5 for a normal D_h chromosome 1 in the 2B-1 translocation configuration lowered the chiasma frequency little or not at all in the left arm (at position a), but appreciably in the right arm (at position b). The evidence of interference in the right arm between chiasmata proximal and distal to the point of translocation (positions b and c) does not alter the conclusion that the D_5 chromosome differs in some way from the D_h chromosome in the right arm, and that the difference is in the direction of less frequent chiasma formation when the two are present together. It does, however, introduce a doubt as to whether the *lack* of difference between the D_5 and D_h left arm is real, or only apparent because of a lack of competition. If a parallel experiment were devised in which the left arm of the D_h chromosome bore a translocated segment, a greater degree of difference might become apparent. On the other hand, the chromosomes of D_5 do pair regularly with those of D_h, hence are assumed to have a high degree of homology with them, and it is not unlikely that such differences as do exist may be unevenly distributed along the various chromosome arms.

STEPHENS (1949, 1950) has suggested that cryptic structural differences (STEBBINS 1945; STEBBINS, VALENCIA and VALENCIA 1946) exist between the chromosomes of closely related genomes of Gossypium species whose F_1 hybrids show no gross structural differences or marked non-homology at meiotic metaphase. A critical test of this hypothesis has not been readily forthcoming. BROWN (unpublished) has shown that pachytene pairing cannot be considered a criterion of chromosome or genome differentiation in Gossypium. Pachytene pairing was as intimate in both sterile and fertile F_1 species hybrids as within species, and was equally complete in all hybrids examined regardless of the degree of metaphase association. BROWN concludes, therefore, that the number of chiasmata persisting until metaphase I is still the best criterion of similarity between different genomes. The pertinent conclusion from the present data would seem to be that chromosome 1 of D_5 is not perfectly homologous with chromosome 1 of D_h, despite the fact that they were paired with each other at metaphase I in all but one out of 390 cells examined. In a formal sense, these data may be considered as evidence for cryptic structural differences between the

chromosomes of the two species, although the real nature of the differences is still obscure. If chiasmata represent crossovers, we may conclude, as STEPHENS (1949) has suggested, that the effect of the differentiation, when the two are combined in a hybrid, will be to reduce crossing over in the chromosome segments concerned, with consequent tendency of the parental characteristics determined by genes on those segments to remain more tightly linked than would be expected within either species. With appropriate translocation lines, it should be possible to test in a similar manner the differentiation of each D_h chromosome from its homologues in D_5 and in several other D genomes.

A low incidence of III's due to DDA pairing rather than to the translocation was found in the *hirsutum-raimondii* hybrid. It was mentioned above that intergenomic pairing was unexpectedly high in (AD)AD three-species hybrids. It has been suggested (MENZEL and BROWN 1954) that this pairing is not at random, but rather confined to specific chromosomes. Some 63 instances of AD chiasmata were found in the 390 cells examined (usually only one, with a maximum of three, per cell). Had any of these been chiasmata between chromosome 1 and an A_h chromosome, the resulting IV would have been readily detectable, but no such configuration was found. Hence it may be said that if this chromosome ever pairs with an A_h chromosome, it does so very infrequently.

The present studies illustrate only two of the ways in which the cytogenetic anatomy of Gossypium genomes may be explored and compared by the use of morphologically marked chromosomes. Further progress will be limited by the ease with which other lines having the morphological convenience of 2B-1, or producing a similar array of deficiency-duplication genotypes, or both, can be obtained. Earlier work (BROWN 1949) and preliminary experiments with X-ray treatments to be reported elsewhere indicate, however, that 2B-1 is not unique in either respect.

SUMMARY

The 2B-1 translocation in *G. hirsutum* consists of an interchange between a very short segment of a chromosome in the D_h subgenome (chromosome 1) and a long segment from a chromosome in the A_h subgenome (chromosome 2).

Chromosome 1 of *G. raimondii* (D_5 genome) shows a reduced frequency of metaphase chiasmata with its *hirsutum* homologue in the right arm but not in the left arm.

Chromosome 2 proved to be one of the 4 A_h chromosomes which differ from the A_1 genome (*G. herbaceum*) in end arrangement. It was demonstrated that when one of the A_h-A_1 IV complexes is introduced into *G. hirsutum* at least two types of deficiency-duplication gametes function in addition to the two types of balanced gametes.

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