THE SIGNIFICANCE OF MULTIVALENT FORMATION IN THREE-SPECIES GOSSYPIUM HYBRIDS

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FOR some years the cotton improvement program at the Texas Agricultural Experiment Station has included studies of three-species hybrids combining tetraploid New World cultivated cottons (with the genome constitution 2(AD)), Asiatic cultivated cottons (diploids of genome constitution 2A) and American wild diploid Gossypiums (2D). A fuller explanation of genome symbols, adapted from BEASLEY, is given by BROWN and MENZEL (1952b).

Earlier several investigators, including the authors, had studied the pairing relations between chromosomes of the 2A and 2D genome groups and 2(AD), but attention had been focused mainly on the completeness or incompleteness of pairing of the diploid genomes with one or the other subgenome of the tetraploid species. This work had established that at metaphase I, chromosomes of 2A species are usually completely paired with 13 chromosomes of the tetraploids, and that the other 13 chromosomes pair completely with those of the 2D species.

In the course of a study of chromosome pairing in three-species hybrids of constitution (AD)AD, in which pairing was therefore expected to approximate 26 II, it was found that modally the chromosomes did indeed show complete pairing, but that there was a high frequency of multivalent formation (BROWN and MENZEL 1950). This led to a re-examination of the literature and reanalysis of hybrids at hand of the constitution (AD)A and (AD)D. The viable (AD)D hybrids between tetraploid species and American wild diploid species all form approximately 13 II and 13 I, with an occasional III presumably composed of a D pair and an A chromosome (for summary see BROWN and MENZEL 1952a). But the $(AD)_1A_1$ *hirsutum-herbaceum* hybrid was found to have modal pairing of 9 II 2 IV 13 I while incomplete analysis of $(AD)_1A_2$, *hirsutum-arboreum,* suggested a mode of 1 IV + 1 VI per cell in addition to II's, I's and III's. Meanwhile GERSTEL (1953) reexamined the pairing relations among *G. hirsutum, G. arboreum* and *G. herbaceum* and concluded that *G. arboreum* and *G. herbaceum* differ by one reciprocal translocation (form 1 IV in the F_1 hybrid), *G. herbaceum* and *G. hirsutum* by two translocations (2 IV), and *G. arboreum* and *G. hirsutum* by three translocations (1 IV + 1 VI). The chromosome end arrangement of *G. herbaceum* was considered the most primitive because it was shown to be identical with that of the primitive wild species *G. anomalum.* GERSTEL has reviewed the

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earlier literature on (AD)A pairing. Some investigators had noted multivalent formation, but at that time evidence was being marshalled to show that the New World tetraploids are of amphiploid rather than autoploid origin, and the full significance of the multivalents was not emphasized. It was not then certain whether they were due to residual autoploidy within the A genome or to segmental interchange. Although BEASLEY (1942) referred to them as translocations, he failed to distinguish between the two IV's of A_1A_h and the IV + VI of A_2A_h.

THEORETICAL CONSIDERATIONS

GERSTEL's study makes it seem certain that the multivalents are due to segmental interchange rather than to polyploidy or duplication within the A genome. This interpretation is supported by the fact that multivalents are extremely rare in normal *G. hirsutum*. No convincing cases have been seen in this laboratory among several thousand pollen mother cells examined each year. Moreover, when the multivalents are introduced into fertile tetraploid plants, segregation for the presence and number of multivalents occurs in their progenies, as is expected in progenies of translocation heterozygotes. If the line had *G. herbaceum* as the A-genome ancestor, plants are recovered with 26 II, 24 II 1 IV, and 22 II 2 IV. If *G. arboreum* was the A-genome ancestor, plants with 26 II, 24 II 1 IV, 23 II 1 VI, and 21 II 1 IV 1 VI are obtained. That is, the two different multivalent figures segregate independently as would be expected if they were due to translocations.

The end arrangements of the five A chromosomes concerned in the translocations, as they are understood at present from all the available evidence, are represented schematically in figure 1. Assuming an initial end arrangement identical with A_1, the cytological configurations cited for A_1A_2, $(AD)A_1$ and $(AD)A_2$ can be explained simply by assuming that one reciprocal translocation occurred and became established in the evolution of A_2, and two reciprocal translocations in A_h. Divergence from the primitive end arrangement of A_1 must have occurred independently in A_2 and A_h, and to obtain the hexavalent in $(AD)A_2$ it is necessary only that one chromosome involved in A_h be one of the chromosomes involved in the A_2 translocation. The remaining eight chromosomes of all the A genomes presumably have identical end arrangements and are not shown.

We may now consider what the cytological effect of the different end arrangements of the various A genomes will be when they are combined in fertile tetraploid hybrids of the general genome constitution 2AD. Any such hybrid which has a complete set of A_h and a complete set of A_1 or A_2 chromosomes should show the same chromosome configurations as the corresponding triploid hybrid, except that the D genome I's of the triploid will now be represented by II's. Hence an $(AD)_1A_1D$ hybrid should characteristically form 22 II 2 IV at metaphase I, and an $(AD)_1A_2D$ hybrid, 21 II 1 IV 1 VI.

In practice, 2AD plants of hybrid origin have been obtained in three ways: (1) by repeatedly backcrossing a colchicine doubled (hexaploid) hybrid,

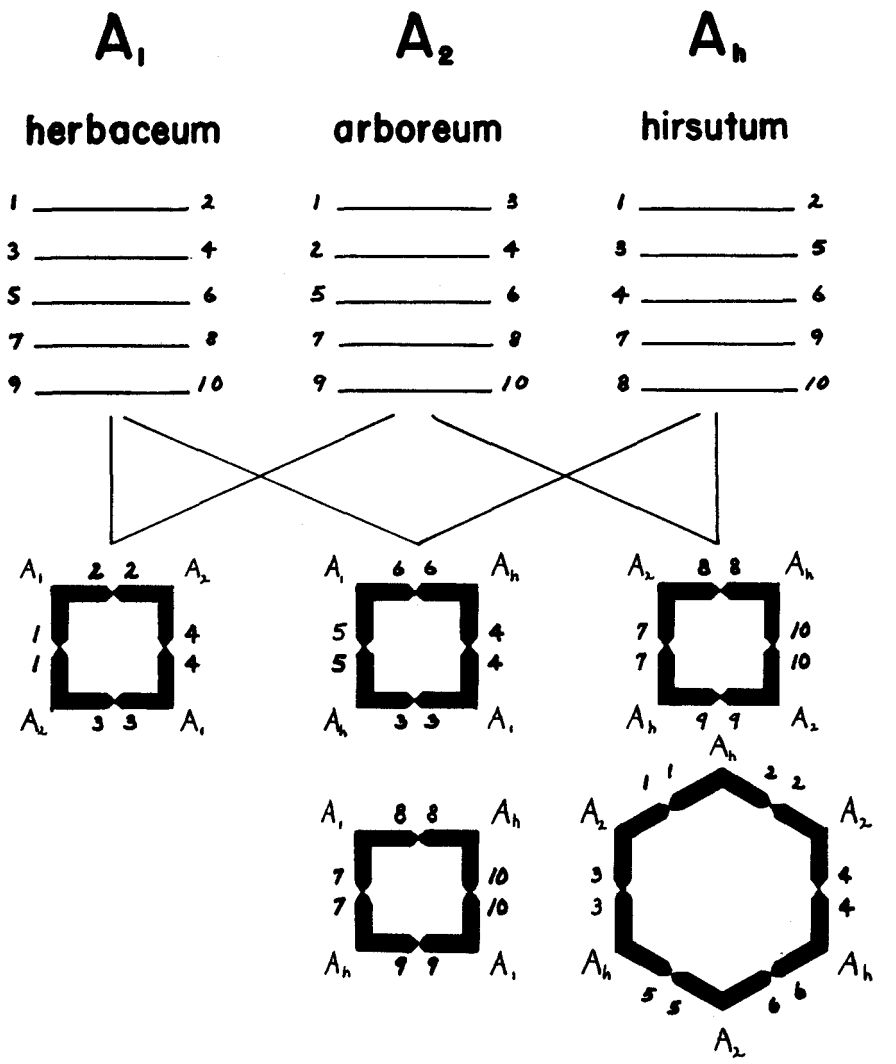

FIGURE 1.—Diagram showing the end arrangements of the differential chromosomes in *G. herbaceum*, *G. arboreum* and the A_h subgenome of *G. hirsutum*.

$2(AD)_1A, \times 2(AD)_1$ until the tetraploid chromosome number is restored (usually by about the third or fourth generation); (2) by crossing a synthesized 2AD allotetraploid $\times$ 2(AD); and (3) by crossing a $2(AD)_1A$ hexaploid $\times$ a 2D species. The first method combines only two species; the second and third method, three species.

When the 2AD hybrid is obtained by crossing synthesized $\times$ natural allotetraploid (method 2 above), it can be assumed, disregarding rare gametes resulting from crossovers between A and D chromosomes, that all of the F_1 hybrids have similar A-genome constitutions of 13 A_h and 13 A_1 or A_2 chromosomes. Hence all should show the full complement of multivalents due

to heterozygosity for the different A-genome end arrangements, and a maximum of 2 IV's per cell in $(AD)_1A_1D$ and of 1 IV + 1 VI per cell in $(AD)_1A_2D$ can thus be attributed to the A-genome translocations.

The situation becomes more complicated when the tetraploid hybrid is obtained by crossing a 2(AD)A hexaploid either to 2(AD) or to 2D species (methods 1 and 3). In this case, both the A_h and A_1 or A_2 components are introduced via the same gamete, i.e., are derived from the same hexaploid meiotic division. The high frequency of multivalents in these hexaploids (for review see BROWN and MENZEL 1952b) indicates that preferential pairing is not very strong in them. Therefore, although their gametes will carry approximately 13 D and 26 A chromosomes, the latter will not be composed of 13 A_h and 13 A_1 or A_2 chromosomes, but of various recombinations of these. Even if only gametes which are balanced for the various ends are functional, three conditions are possible for each of the two groups of differential chromosomes: homozygous for A_h end arrangements, homozygous for A_1 or A_2 end arrangements, or heterozygous. Since the two groups segregate independently of each other, nine different kinds of gametes are possible from either $2(AD)_1A_1$ or $2(AD)_1A_2$ if all of the differential chromosomes are considered. If duplications and deficiencies are viable, this number will of course be increased.

Therefore, the F_1 hybrids having a hexaploid as one parent will differ from each other in their A_h and A_1 or A_2 composition unless there is very strong selection for a single kind of functional gamete in the hexaploid. This point may be tested by comparing the multivalent configurations in individual F_1 plants and in their first backcrosses to *G. hirsutum*.

Data have accumulated over a period of years on metaphase pairing in hybrid material obtained by all three of the methods considered above. They will be examined here with a view to answering the following questions: (a) Can the A_1 and A_2 end arrangements be transferred to an essentially *hirsutum* background? (b) Can all of the multivalent formation in $(AD)_1AD$ three-species hybrids be attributed to the A-genome translocations? (c) Do three-species hybrids obtained via hexaploid × diploid crosses differ from each other and from hybrids from tetraploid × tetraploid crosses with regard to the A-genome chromosomes involved in the translocations? (d) What is the behavior of the multivalents in subsequent generations, and what bearing may they be expected to have upon the breeding behavior of species-hybrid derivatives?

MULTIVALENTS FROM HIRSUTUM-ASIATIC PENTAPLOIDS

Data from backcrosses of the *hirsutum*-Asiatic hexaploids to *hirsutum* were collected incidental to recovery and study of trisomic lines and without particular effort to isolate or maintain the translocations. Reexamination of these data allows the following conclusions, however: (1) All of the multivalent formation in the pentaploid and later generations (above that attributable to aneuploidy) can be accounted for by the A-genome translocations. No pairing

between A and D genomes need be postulated. (2) Lines carrying single translocation multivalents which subsequently behave cytologically as if they were reciprocal translocations within *G. hirsutum,* are readily isolated in plants having only 52 chromosomes. As cytogenetic tools, these lines have the advantage over induced translocations in *G. hirsutum* that they are already known to involve the A_h rather than the D_h subgenome. Several such lines from both *arboreum* and *herbaceum* have been established and are being tested against each other and against cytologically aberrant *G. hirsutum* lines to establish whether the same or different chromosomes are involved.

MULTIVALENTS IN TETRAPLOID × TETRAPLOID THREE-SPECIES HYBRIDS

Metaphase I pairing in the *arboreum-thurberi-hirsutum* hybrid, A_2D_1-$(AD)_1$, was previously reported in some detail (BROWN and MENZEL 1950, table 1). Reference to this report shows that only two out of 58 PMC's analyzed showed the expected pairing of 21 II 1 IV 1 VI. Two modes lay at 23 II 1 VI and 24 II 1 IV (5 and 6 cells respectively). Since it is not expected that all of the possible chiasmata will form in every cell in a translocation heterozygote, multivalent formation of 1 IV + 1 VI *or less* can be considered to be due to the A-genome translocations. Only 33 out of the 58 cells analyzed can be so accounted for. The remaining cells show evidence of excess multivalent formation. There is no evidence that any of the D genomes differs structurally from the D_h subgenome in such a way as to give multivalents. But there is considerable evidence that varying amounts of residual homology exist between various A and various D genomes. Metaphase pairing ranges from an average of 1.5–1.7 associations of two chromosomes per cell in *G. hirsutum* haploids (BEASLEY 1942; BROWN and MENZEL 1952a) to about 4.7 (BEASLEY 1942) to 8 (SKOVSTED 1937) in A_2D_1 F_1 hybrids. Hence it may be concluded that excess multivalent formation is due to chiasmata between A and D chromosomes. This conclusion is supported by the appearance of some of the multivalents at metaphase I. The A chromosomes are on the whole somewhat larger than the D chromosomes. Although this size difference is neither large enough nor constant enough to be used as a precise criterion in all preparations, it can often be used as an indication of the genome affinity of a chromosome or group of chromosomes. Notes taken during study of the $A_2D_1(AD)_1$ hybrid, before the present hypothesis regarding multivalent formation was formulated, indicated that many of the multivalents were composed entirely of large chromosomes, but that some were composed partly of large and partly of small chromosomes.

In the $A_2D_1(AD)_1$ hybrids, not more than three chiasmata between A and D chromosomes need be postulated to account for pairing in any of the cells recorded; 18 of the 25 cells can be accounted for by assuming only one AD chiasma. It is of interest that this frequently seems to involve pairing between a D II and the A_hA_2 VI. In 10 of the 25 cells, either an VIII, a VII + I, or 1 III + 1 V without I's was formed. Some of the other configurations may also be explained in this way if the potential VIII of 6 A and 2 D chromosomes

forms, instead, 2 IV's, 1 III + 1 IV + 1 I, etc. The presence of an association of 10 chromosomes in one cell suggests that the D II also has some homology with one of the A_hA_2 II's, perhaps in the opposite arm. The frequent involvement of the VI in AD pairing suggests that the intergenomic pairing is not at random, but rather confined to only a few segments of the D genomes.

MULTIVALENTS IN HEXAPLOID × DIPLOID THREE-SPECIES HYBRIDS

In the previous report of pairing in *hirsutum-arboreum-harknessii,* $(AD)_1A_2D_{2\text{-}2}$, and *hirsutum-herbaceum-harknessii,* $(AD)_1A_1D_{2\text{-}2}$ (BROWN and MENZEL 1950), data from several plants were combined. Sufficient cells from five different hexaploid × diploid F_1 plants have been analyzed to allow them to be considered separately (table 1). In all five of these plants, the average number of chromosomes per cell participating in multivalent formation was lower than in the hybrids obtained by tetraploid × tetraploid crosses. In all five, however, some cells showed multivalents in excess of those ascribed to A-genome translocations, ranging from only one cell (2.5%) in one $(AD)_1A_2D_{2\text{-}2}$ hybrid to 36% of the cells in the $(AD)_1A_1D_{2\text{-}2}$ hybrid. In all five, the estimated frequency of AD pairing was lower than in the $A_2D_1(AD)_1$ hybrids.

The lower multivalent frequency in the five hexaploid × diploid hybrids suggests that none of them was heterozygous for all of the chromosomes involved in the translocations.

MULTIVALENTS IN F_2 AND BACKCROSSES FROM THREE-SPECIES HYBRIDS

A summary of pairing in first backcross progenies of two different hexaploid × diploid $(AD)_1A_2D_{2\text{-}2}$ hybrids is shown in table 2. Plants from F_1 Z593 (which was not analyzed) probably all had the IV (one plant was doubtful), while four did and three did not have the VI. Plants from Z595 × *hirsutum* all exceeded Z595 in multivalent formation, and of 21 plants, all but one or two had the IV, and all had the VI, despite the fact that only two VI's were seen in 25 PMC's of the parent. These findings indicate that Z593 and Z595 did not have identical A_2A_h constitution, and that Z595 at least was probably a duplication-deficiency type which had become homozygous for one or more of the A_2 end arrangements. (Had it been homozygous for A_h end arrangements, multivalent formation should not have increased on backcrossing to *G. hirsutum*). Also of interest is the fact that some of the first backcross plants exceeded their parent in frequency of AD chiasmata, and approached or equaled the $A_2D_1(AD)_1$ hybrids in frequency and complexity of multivalent formation. It will be noted that apparently none of the 28 first backcross plants had returned completely to the *G. hirsutum* end arrangement. All showed at least one translocation configuration.

Unfortunately, insufficient first backcross plants from tetraploid × tetraploid $A_2D_1(AD)_1$ have been analyzed to permit a direct comparison. Table 3, however, summarizes the pairing in 16 F_2 plants from this hybrid. Seven to ten plants had both IV and VI, two had only the VI, three only the IV, and one trisomic plant had neither. With the exception of one plant from which

TABLE 1

Comparison of metaphase pairing in three-species Gossypium hybrids of genome constitution (AD)AD.

Plant No.	Type of cross	Genome symbol	No. PMC	Av. No. chromosomes per cell paired as multivalents[b]	Cells with excess multivalents		AD chiasmata per cell	
					No.	%	Mode	Max.
Combined data, several plants	Tetraploid × tetraploid	$A_2D_1(AD)_1$	58	9.80	25	43.1	1	3
Z905	Hexaploid × diploid	$(AD)_1A_2D_1$[c]	11	7.45	3	27.2	1	2
Z594	Hexaploid × diploid	$(AD)_1A_2D_{2-2}$	42	5.74	1	2.5	1	1
Z595	Hexaploid × diploid	"	25	4.64	4	16.0	1	1
Z596	Hexaploid × diploid	$(AD)_1A_1D_{2-2}$	25	5.76	9	36.0	1	4
Z907	Hexaploid × diploid	$(AD)_1A_2D_5$[a]	28	7.89	4	14.3	2	2

[a] D_5 = *G. raimondii* genome.
[b] Including trivalents.
[c] Hybrid made by J. R. Meyer.

Pairing in the first backcross generation from two different $(AD)_1A_2D_{2-2}$ F_1 hybrids × G. hirsutum.

Parent	Plant No.	Direction of cross[a]	No. PMC analyzed	A-genome translocation multivalents present		AD pairing		
				IV	VI	No. cells	%	Max. AD Xmata
A. Z593	52C-1-1950	♂ ♀	38	+	+	2	5.2	2
	52H-3-1950	♀ ♂	35	+	+	10	28.5	2
	426B-2-1951	♀ ♂	17	+	+	3	17.6	2
	Z869	♀ ♂	22	?	+	?	?	?
	Z868	♂ ♂	17	+	....	....	....	....
	52C-3-1950	♂ ♂	10	+	....	4	40.0	1
	52C-4-1950	♂	20	+	....	....	....	....
B. Z595	Z755	♀	26	+	+	1	3.8	1
	Z756	♀ ♀	33	+	+	....	....	....
	Z759	♀ ♀	29	+	+	2	6.9	1
	Z760	♀ ♀	28	+	+	6	21.4	1
	Z761	♀ ♀	25	+	+	3	12.0	1
	52E-1-1950	♂ ♀	34	+	+	3	8.8	1
	52F-1-1950(2n + 1)	♀ ♀	33	+	+	?[b]	?[b]	?[b]
	52F-2-1950	♀ ♀	25	+	+	8	35.0	1
	52G-2-1950	♀ ♀	30	+	+	3	10.0	1
	52G-3-1950	♀ ♀	25	+	+	2	8.0	1
	52I-1-1950	♀ ♀	18	+	+	6	33.3	1
	52I-2-1950	♀ ♀	26	+	+	8	30.8	2?
	52I-3-1950	♀ ♀	17	+	+	2	11.7	1
	53A-1-1950	♀ ♀	20	+	+	4	20.0	1
	53A-2-1950	♀ ♀	28	+	+	5	17.7	3
	Z891	♀ ♂	33	+	+	11	33.3	2
	426A-2-1951	♂ ♂	8	+	+	2	25.0	1
	426A-4-1951	♂ ♂	31	+	+	....	....	....
	426C-1-1951	♀ ♀	9	+	+	....	....	....
	Z757	♀ ♀	22	?	+	....	....	....
	52G-1-1950[c]	♀	23	....?	+?	1	4.3	1

[a] Symbol indicates whether the F_1 hybrid was ovule or pollen parent.

[b] This 53-chromosome plant gave complex pairing, one cell showing maximum pairing of 1 III, 1 IV, 1 chain of X.

[c] This plant probably lacked the IV and had a duplication-deficiency from the VI. It showed modal pairing (11 cells) of 1 IV + 2 I, but some cells had a VI.

TABLE 3

Pairing in F_2 plants from arboreum-thurberi-hirsutum.

Plant No.	No. PMC	IV	VI	AD pairing		
				No. cells	%	Max. AD Xmata
139B-2-1949	16	+	+	2	12.5	1
140A-6-1949(2n + 1)	13	+	+	3	23.0	4
141A-6-1949	15	+	+	2	13.3	1
141A-7-1949(2n + 1)	8	+	+	1	12.5	1
194A-3-1949	26	+	+	10	38.4	4
292A-19-1949(2n + 1)	19	+	+?	....	....	....
Z696	20	+	+	5	25.0	1
Z697	25	+	+	8	32.0	2
140A-2-1949	9	+?	+	?	?	?
194A-6-1949(2n + 1)	24	+?	+	....	....	....
141A-3-1949	12	....	+	4	33.3	1
292A-13-1949	5	....	+	5	100.0	1
141A-13-1949	56	+	....	6	10.7	1
194A-4B-1949	6	+	....	....	....	....
291B-13-1949	36	+	....	5	13.9	1
292A-15-1949(2n + 1)	28	....	....	....	....	....

only five cells were analyzed (four having 22 II 1 VIII and one, 22 II 2 IV), all the F_2 plants had a lower frequency of cells with AD chiasmata than the F_1. Since the backcross plants listed in table 2 indicate that both the A_h and A_2 end arrangements may pass through both pollen and ovules, it should be pointed out that in the F_2, when one of the translocation configurations had been lost, the plant may have become homozygous for either the A_h or the A_2 end arrangement.

DISCUSSION

Experience with *hirsutum-barbadense* and with three-species hybrids in Gossypium, recently reviewed by RICHMOND (1951), has shown that when species hybrids are incorporated into a breeding program, certain difficulties are encountered which are not present in intraspecific breeding. These difficulties do not indicate that such programs cannot succeed, but rather that much more information is needed concerning the nature of the barriers which interfere with free recombination of desirable traits and the ready transference of species-foreign characters to an otherwise *hirsutum* background. At least two hypotheses have previously been proposed to explain these barriers, the " multiple gene substitution " theory of HARLAND (1936) and " cryptic structural hybridity " in the sense of STEBBINS (STEPHENS 1950). With regard to the three-species hybrids, it is now necessary to consider also the possible effects of gross differences in chromosome structure.

We are now in a position to give tentative answers to the questions which were raised above:

(a) The differential chromosome end arrangements of A_1 and A_2 can be maintained indefinitely, at least in heterozygous condition, on an essentially *hirsutum* background.

(b) Data at hand show that the A-genome translocations can account for all the multivalent formation in backcrosses from the *hirsutum*-Asiatic hexaploids (above that due to aneuploidy), and for a major portion, but not all, of that in the three-species hybrids. The excess multivalents in the latter must be attributed to pairing between A and D chromosomes. It was estimated that as high as 43% of PMC's may show from one to four such intergenomic chiasmata. It should be pointed out that this estimate is a minimum value. It is probable that the actual frequency is somewhat higher. The frequent occurrence of AD chiasmata was not expected from what was known concerning (AD)A and (AD)D triploid hybrids, *hirsutum*-Asiatic pentaploids, and preferential pairing in general in the genus.

(c) The available data strongly indicate that F_1 three-species hybrids derived from hexaploid × diploid crosses are not uniform in A-genome composition, and may not be assumed to be exactly equivalent to similar hybrids obtained by crossing synthesized × natural allotetraploids. This in turn suggests that if breeding is to proceed from such hybrids, selection of parents can and should begin in the F_1 generation.

(d) Probably the translocations can persist, unless intentionally selected against, for some time in breeding material, especially if selection for high fertility has not been the main objective. For instance, when a second backcross plant from the *arboreum-thurberi-hirsutum* hybrid, selected for high fiber strength, was selfed, only one out of seven plants analyzed cytologically had 26 II. One still retained the full A_hA_2 multivalent complex of 1 IV + VI, while the others showed at least one multivalent configuration. Thus if a breeding line has been selfed, one is not certain that it is homozygous for the *hirsutum* end arrangements, even though it shows only 26 II at metaphase. And even if it has been backcrossed two or more times, it may not be assumed without cytological confirmation that all the *hirsutum* end arrangements have become homozygous.

Moreover, some of the data suggest that some of the duplication-deficiency gametes from the A-genome translocations may be functional (as they are in at least one induced reciprocal translocation in *G. hirsutum,* MENZEL and BROWN 1952). The existence and significance of these types remain to be verified; if present, they may have considerable bearing on some of the complications that arise in the breeding work.

It is not yet possible to estimate how much the A-genome translocations interfere with fertility in the derivatives of three-species hybrids, but it is likely that they reduce it to some extent, both through non-disjunction and by retaining large unbroken blocks of species-foreign genic material.

In many instances in which the (AD)AD three-species hybrids have been used in breeding programs, the primary intention has been to introduce into *G. hirsutum* characters from the American wild ancestor. The A-genome translocations should not interfere with the transference of chromosome segments from the D ancestor. However, the fairly high frequency of excess multivalents, deduced to be due to AD chiasmata, may do so. Assuming that they represent crossovers, such chiasmata can give rise in backcrosses to

G. hirsutum to a variety of cytological aberrations, including new reciprocal translocations between A and D chromosomes, and possibly also to insertions of D segments into A chromosomes or vice versa, where, with continued backcrossing to *hirsutum* (in which AD pairing does not occur) they may become " trapped " and difficult to alter or eliminate by crossing over.

It appears that the irregularities of meiosis in the F_1 three-species hybrids may be primarily of nuisance value for practical breeding purposes. In this regard, it would be of importance to know how quickly they may be eliminated without also eliminating the species-foreign characters which it is desired to retain. These aberrations are otherwise of interest, however, as a source of cytological types for use in basic study of the comparative cytogenetics of Gossypium species, since they may be expected to include several different kinds of translocations, deficiency-duplications, substitution races, and possibly other types not yet recognized.

SUMMARY

Demonstration that the A_h subgenome of *G. hirsutum* differs from the A_1 (*G. herbaceum*) genome by two, and from the A_2 (*G. arboreum*) genome by three reciprocal translocations led to re-evaluation of multivalent formation in three-species Gossypium hybrids combining *G. hirsutum, G. herbaceum* or *G. arboreum,* and American wild diploid species (D genome). The A-genome translocations account for all the multivalent formation not due to aneuploidy in *hirsutum*-Asiatic pentaploids and their descendants. Excess multivalent formation occurring in the three-species hybrids and their progeny is attributed to chiasmata between A and D chromosomes. As high as 43% of pollen mother cells may show from one to four AD chiasmata at metaphase I. Three-species F_1 hybrids obtained by crossing 2(AD)A hexaploids × 2D species differ from each other both in their complement of A-genome end arrangements and in the frequency of AD chiasma formation. Some first backcross plants from such hybrids exceed their parents in multivalent formation of both types. Implications of A-genome multivalent formation and of AD chiasmata are discussed. Although the A-genome translocations may be recovered in heterozygous condition even after several backcrosses to *G. hirsutum,* they may also be rather quickly eliminated if deliberately selected against. The possible consequences of the rather high frequency of AD pairing remain to be verified. In addition to the hypotheses of " multiple gene substitution " and " cryptic structural hybridity," previously advanced to account for problems peculiar to interspecific breeding in Gossypium, use of the three-species hybrids must also take into account the gross structural differences already existing between the A genomes, and the possibility of newly-arising rearrangements resulting from chiasmata between A and D chromosomes.

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