VIABLE DEFICIENCY-DUPLICATIONS FROM A TRANSLOCATION IN GOSSYPIUM HIRSUTUM [1]

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GOSSYPIUM hirsutum L., which includes the Upland varieties of cultivated cotton, is known to be an allotetraploid species with 26 pairs of chromosomes. Thirteen relatively large pairs (A genome) are closely homologous with those of the diploid Asiatic cultivated cottons, and thirteen relatively small pairs (D genome) with those of wild New World diploid species of Gossypium (for recent review see BROWN 1951). Several pairs of genes are known to be linked in *G. hirsutum*, but these have not been assigned to specific chromosomes. Beyond this, little is known of the cytogenetic anatomy of *G. hirsutum*. At present, an attack on this problem is being made by various methods, including cytological and genetic studies of trisomic, tetrasomic, monosomic and translocation lines.

The present paper deals with a translocation from which not only normals and heterozygous translocations are recovered on backcrossing to normal lines, but also an array of duplication and deficiency types. This translocation is of interest not only because of its cytological behavior, but also because some of its derivatives should prove useful in further linkage studies.

The original translocation appeared in a plant (designated 2B-1) grown from seeds of a commercial variety of cotton, Acala 911, which were exposed to gamma radiation of unknown intensity and duration at the time of the explosion of the first atomic bomb at Bikini (Operation Crossroads). This material was obtained through the courtesy of the U. S. Department of Agriculture in cooperation with the Naval Medical Research Section, Joint Task Force One.

Cytological analyses were carried out for the most part on field-grown plants, and selected plants were transplanted to the greenhouse for crossing and selfing. Buds were fixed in a mixture of 3 parts glacial acetic acid: 7 parts 95 percent alcohol, and pollen mother cells were smeared in iron-acetocarmine for the study of meiosis. Figures for frequencies of different meiotic configurations are taken from 1951 analyses only.

THE HETEROZYGOUS TRANSLOCATION

A description of chromosome configurations in 2B-1 is given by BROWN (1950). Configurations in recovered heterozygous translocations agree with

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this description. Figure 1 shows the frequency of the various configurations and diagrams of their presumed structure. So far as can be seen at metaphase I in the pollen mother cells (the earliest stage of meiosis which can be studied readily in cotton), the translocation behaves as if it were nonreciprocal. Most of the long arm of a large chromosome with subterminal centromere (chromosome 2) appears to have been translocated terminally to one arm of a second large chromosome with approximately median centromere (chromosome 1). The characteristic configuration is a bivalent with an attached chain of two. A chiasma is usually formed proximal to the point of attachment of the long fragment (designated "A" for "attached fragment"), and the broken arm of the centric fragment ("C") is always free. Since there is no evidence that broken ends of chromosomes ever unite with unbroken ends, it must be assumed that a minute terminal segment of chromosome 1 (designated "x") was broken off, but we have at present no cytological evidence as to whether x was translocated to the broken end of C, or lost, which would require healing of the broken end of C (see McCLINTOCK 1941).

For descriptive purposes, therefore, it will be convenient to treat the translocation as if it were nonreciprocal, and to ignore the questionable status of the x segment. Some indirect evidence suggests, however, that the x segment contains functional chromatin, and that the translocation is actually reciprocal but very unequal.

Plants heterozygous for the translocation are normal in appearance, so that cytological analysis is necessary for their identification. No controlled comparison of their fertility with that of normal plants has been undertaken, but well-filled bolls are set following both self and cross pollination, indicating that at least ovule fertility is approximately normal. The studies reported here were carried out in crosses using the translocation heterozygote as the pistillate parent; hence pollen fertility has not been tested.

Recovery of deficiency and duplication types

The original translocation (2B-1) was brought into the greenhouse in 1947 and allowed to set seed. In 1949 a progeny of 13 plants was grown from the seed, of which 11 were examined cytologically. In addition to normals and translocation heterozygotes, several plants appeared which could not be assigned with confidence to either of these classes. However, it was thought possible, though unlikely, that 2B-1 was a chimera of several different cytological types produced by the radiation (BROWN 1950). Two plants with heterozygous translocation configurations of the 2B-1 type, and a third unanalyzed plant (subsequently shown to have been of the same type) were crossed as pistillate parents with a cytologically normal genetic marker stock (SL7-9) carrying five independent dominant characters.

Small progenies were grown from these crosses the following summer, and meiosis was studied in fifteen plants. It was again apparent that in addition to normals and translocation heterozygotes, other cytological types were being recovered. The extreme inequality of the interchanged arms fortunately made

it possible to distinguish each of these types with ease at metaphase I. The meiotic configurations of 14 of the plants agreed with those expected if maternal gametes were being recovered from both disjunction and nondisjunction of the translocation IV. The fifteenth plant was one of the types expected if a maternal gamete resulting from numerical (3 : 1) nondisjunction were recovered. These results, the gametes involved, the identifying meiotic configuration, and the designation adopted for the resulting genotype are shown diagrammatically in figure 2.

One plant was analyzed from a selfed progeny of a fourth plant of the 1949 progeny heterozygous for the same translocation. This plant had 26 II plus a minute I. Since this type is also expected from numerical nondisjunction of the IV, the plant was considered to be of the constitution $2n + C$.

Two of the heterozygous translocations recovered in 1950 were outcrossed to recessive normal lines, and 68 plants were grown from these crosses in 1951. Meiosis was studied in 58 of these. The types found agreed with those of 1950, with two exceptions: (1) The $2n - C$ and $2n + C$ types did not reappear. (2) One plant of the $2n + A$ type was monosomic for one chromosome not involved in the translocation. This plant will be ignored in the present discussion. The distribution of the remaining 57 plants among the four expected types is shown in figure 2. The frequency of the four types (26 II, IV, $2n - A$, $2n + A$) did not differ significantly from equality ($\chi^2 = 1.88$, $P = 0.50 - 0.70$, 3 d.f.).

Cytological considerations

The majority of heterozygous translocations studied in plants have been characterized by partial sterility, in most cases approximating 50 percent (cf. RILEY 1948, pp. 402–411). This result is generally attributed to nonfunctioning of the gametes (duplications, deficiencies, or both) resulting from nondisjunctional separation of the chromosomes at meiosis. Deviations above or below the 50 percent level of fertility have generally proved to be due to a preponderance of metaphase I orientations on the spindle resulting in an excess of disjunctional or nondisjunctional separations, respectively.

The present translocation differs markedly from the usual case, therefore, since at least the female gametes resulting from disjunction and from nondisjunction are recovered with approximately equal frequency. This implies either that (1) there is a preponderance of nondisjunctional separations but selection favors the disjunctional gametes to just such an extent that they are recovered as frequently as the $n - A$ and $n + A$ gametes, or (2) that there is no selection against $n - A$ and $n + A$ gametes and the meiotic orientation assures random assortment of the chromosome segments involved.

A study of pairing and orientation at metaphase I in 139 pollen mother cells (combined data from 11 heterozygous translocation plants) indicates that the latter alternative is the correct explanation. The data are shown in figure 1. It is deduced from these data that the equality of disjunctional and nondisjunctional gametes ensues from the following considerations. The break in the

arm of chromosome 2 which led to the C fragment was close to the centromere, proximal to the segment in which chiasmata are formed. In 566 cells analyzed from 59 $2n - A$ plants, as well as in the 139 from the heterozygous translocation, the broken arm of C was always free. But the unbroken arm of C was paired with the short arm of chromosome 2 about 79 percent of the time (fig. 1 a, b, c, d, f). Hence if C and chromosome 2 disjoin at all, the first division must be reductional for the broken arm as well as for the centromeres. On the other hand, the point of attachment of A on chromosome 1 appears to be distal to the chiasma-forming region. In 98.65 percent of the cells (all but two, fig. 1 c, d) an interstitial chiasma was present in this arm. As pointed out by SANSOME (1933) and SUTTON (1935), this assures that the first division is almost always equational for the A segment, and the second division

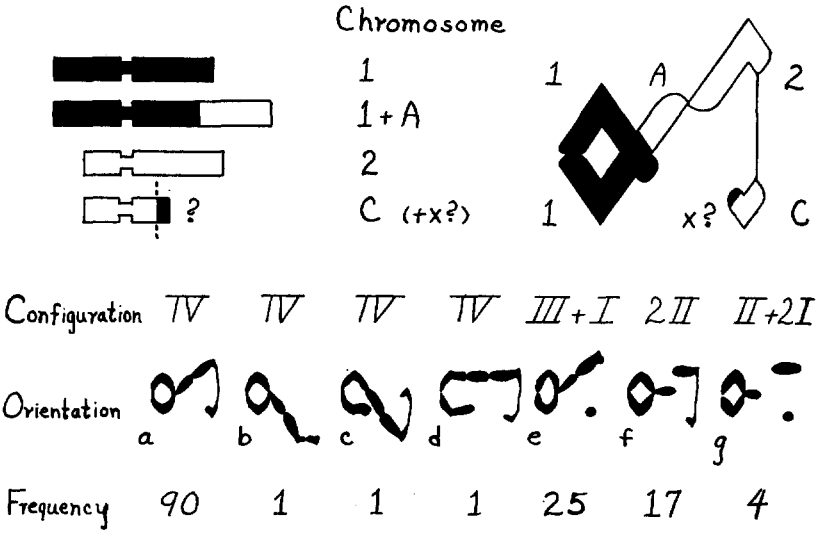

FIGURE 1.—Structure of the heterozygous translocation and frequencies of the various orientations observed at metaphase I in 139 pollen mother cells (1951).

reductional (assuming that the single visible chiasma represents one and only one crossover).

All individual meioses in which the first division is reductional for C and equational for A would result in two " disjunctional " and two " nondisjunctional " gametes, regardless of the orientation of the centromeres at metaphase I. In the sample studied, 107 cells (76.3 percent) fulfilled these requirements (fig. 1 a, f).

If C and chromosome 2 pair but no chiasma is formed between A and the centromere of chromosome 1, the first division is reductional for both A and C, and disjunction depends upon the metaphase I orientation of the centromeres of chromosome 2 with respect to those of chromosome 1. Two cases of this sort were seen in the heterozygous translocations. In one (fig. 1 c), separation was proceeding disjunctionally, and in the other (fig. 1 d), nondisjunc-

tionally. Hence, a total of 109 cells (78.4 percent) recorded should give a 1 : 1 ratio of disjunctional and equal nondisjunctional gametes.

The remaining 30 configurations (21.6 percent) assure ultimate disjunction of chromosomes 1 and 1 + A, but allow chromosomes 2 and C to pass to either the same or opposite poles (fig. 1 b, e, g). If this occurs at random, half should give disjunctional and nondisjunctional gametes in a ratio of 1 : 1. The other half (excluding loss of the univalent chromosomes) should give only gametes of the numerical nondisjunctional classes ($n-C$, $n+C$, $n+C+A$, $n-1$). These four types of gametes together should therefore constitute about 10.8 percent of the total, while the remaining types should make up about 89.2 per-

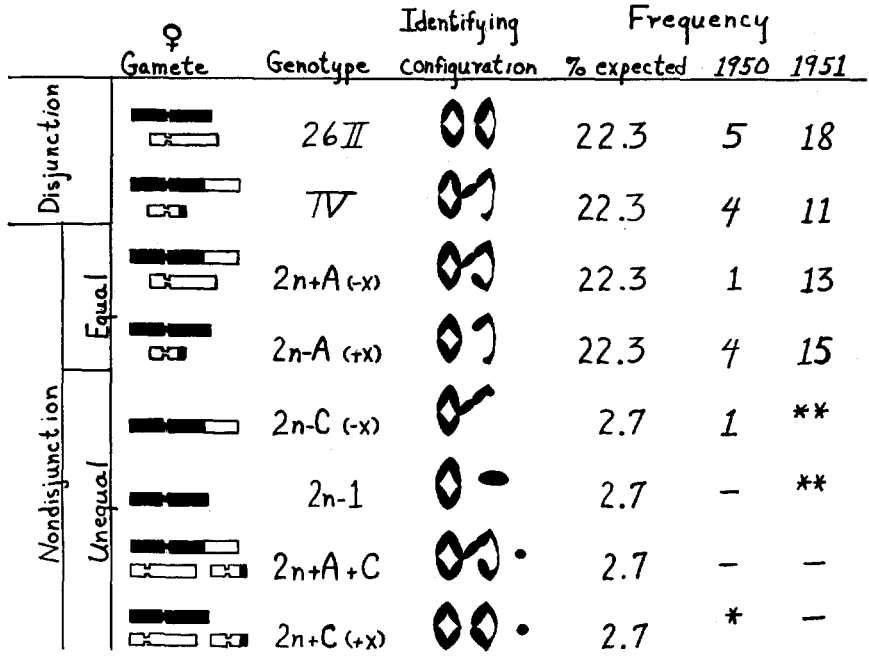

FIGURE 2.—Results of the cross, translocation heterozygote female X normal male. *One recovered from IV selfed. **Recovered from $2n-C$ selfed.

cent of gametes, equally distributed among two disjunctional and two nondisjunctional classes. These expectations are shown in figure 2. It must be pointed out that these studies of meiosis were carried out on pollen mother cells, while the gametes actually tested resulted from meiosis in the embryo sac mother cells. It will be noted, however, that at least the disjunctional and equal nondisjunctional classes expected and recovered are in close agreement. The numerical nondisjunctional gametes have not been recovered quite so frequently as expected from the cytological estimate. Actually only one has been found in backcrosses of the heterozygous translocation to normal lines, among a total of 72 plants, whereas about seven or eight might have been expected. This discrepancy might be due to sampling error, to loss of univalents at anaphase I, to a slightly different pattern of frequencies of the various configura-

tions in the embryo-sac mother cells, or to a combination of these and other factors. It is possible that the numerical nondisjunctional gametes suffer from some selective disadvantage which reduces their rate of recovery. That the zygotes they enter are viable, however, is shown by the fact that three of the four genotypes have been recovered either from the heterozygous translocation itself or from its $2n - C$ derivative (fig. 2).[2] These three types are vigorous and fertile, not phenotypically different from the more common genotypes.

TRANSMISSION OF THE UNBALANCED COMPLEXES

It will be seen from figure 2 that two of the nondisjunctional complexes recovered from the heterozygous translocation are deficiencies for complementary portions of chromosome 2; $2n - A$ is monosomic for most of the long arm, while $2n - C$ is monosomic for the centromere region and the short arm. If the monosomic condition were transmitted, these types could be used in testing for the location of marker genes not only upon chromosome 2, but upon specific portions of it. Cytological classification of the offspring of these and other unbalanced types would also serve as an independent test of our

TABLE 1

Transmission of the $2n - A$ condition.

Types expected	Number of plants analyzed from		
	$2n - A$ selfed	$2n - A♀ \times$ normal	normal $\times$ $2n - A♂$
26 II	51	28	32
$2n - A$	38	7	0
$2n - 2A$	0	0 (not expected)	0 (not expected)
Total	89	35	32

assumptions regarding the constitution of the parent plants, since the cytological classes expected from each are different.

Accordingly, progenies from $2n - A$, $2n - C$ and $2n + C$ plants recovered in 1950 were grown in 1951. Rather extensive analyses of meiosis were made, since information on pairing and metaphase I orientation of the various chromosome segments has important implications for the further use of these types in cytogenetic studies.

Transmission of the $2n - A$ condition

Three of the $2n - A$ plants were selfed, and outcrossed as both seed and pollen parent. As can be seen from table 1, plants deficient for A were readily recovered through the ovule, and no evidence was obtained that $n - A$ pollen functioned. A discrepancy appears when the frequencies of $2n - A$ plants in selfed and outcrossed progenies are compared, however: recovery was appreciably higher in the selfed progeny. No explanation is offered for this fact at present.

[2] In the summer of 1952, the fourth type, $2n + A + C$, was recovered from the heterozygous translocation selfed.

Among 566 pollen mother cells from a total of 59 $2n-A$ plants, the C fragment and chromosome 2 failed to pair 21 percent of the time. Provided the univalents are not lost but separate at random, about 5 percent each of $n+C$ and $n-1$ gametes should be produced by $2n-A$ plants. These types have not been recovered from $2n-A$. It is probable, however, that occasional $2n+C$ and $2n-1$ plants can occur, since both $n+C$ and $n-1$ eggs are known to have functioned in other progenies.

Transmission of the $2n+C$ condition

In 1951 a small progeny was grown from selfed seeds of the $2n+C$ plant. Only ten plants were analyzed, of which six had 26 II and four were $2n+C$. Plants with two C fragments were not found. It is likely that the $n+C$ gametes were recovered from the ovules and that only n pollen functioned, although data from trisomic studies in which tetrasomics were recovered (BEASLEY and

TABLE 2

Transmission of unbalanced gametes from $2n-C$ selfed.

	Constitution of ♀ gamete	Number recovered
Types expected if only n pollen functioned		
26 II	n	15
$2n-C$	$n-C$	9
$2n+A$	$n+A$	7
$2n-1$	$n-1$	7
Other types found (expected if $n+A$ pollen also functioned)*		
$2n+2A$	$n+A$	2 (dwarf)
$2n+A-C$	$n-C$	2 (dwarf)

*Two additional dwarf plants were not analyzed.

BROWN 1943; BROWN 1949, and unpublished) make it probable that $2n+2C$ plants could be obtained occasionally.

In a total of 20 $2n+C$ pollen mother cells, the C fragment was paired in a III 50 percent of the time, in comparison with IV and $2n-A$ plants, in which C was paired 77–79 percent of the time. Although C can pair half the time even when two normal chromosomes 2 are present, no case was seen in which C was paired with one normal chromosome and the other normal chromosome was unpaired. Probably only n and $n+C$ gametes are to be expected from $2n+C$.

Transmission of unbalanced types from $2n-C$

The $2n-C$ plant was selfed and a progeny of 49 plants was grown. Meiosis was studied in 42 of these. Of the analyzed plants, 38 conformed cytologically with the types expected if all four kinds of female gametes (n, $n-C$, $n+A$, $n-1$) functioned and were fertilized with normal n pollen (table 2). Although there was a preponderance of normal (26 II) types, it will be seen that the

other three classes were recovered with appreciable frequency. As in progenies from the heterozygous translocation and from $2n-A$ plants, the cytologically unbalanced plants were phenotypically normal and fertile.

Six plants in the progeny of 49 plants did differ morphologically from the rest, however. These were very small and slow growing, with slender stems reaching a maximum height of less than a foot during the growing season. One succumbed after setting a small boll. The other five were transplanted to

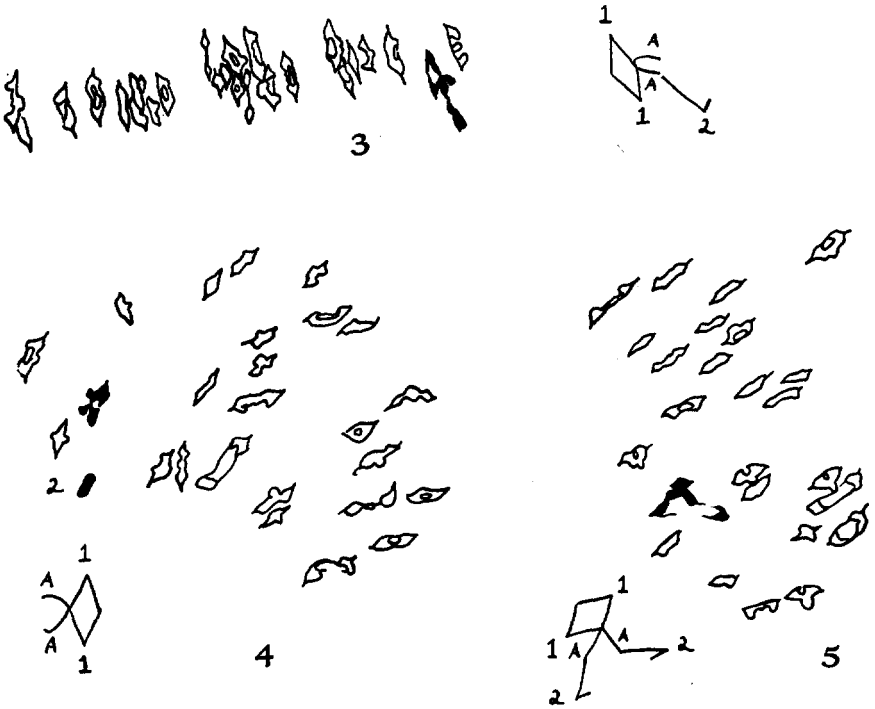

FIGURE 3.—Metaphase I in a dwarf, $2n+A-C-2x$ plant, showing III composed of two $1+A-x$ chromosomes and one chromosome 2.

FIGURE 4.—Metaphase I in $2n+A-C-2x$ plant, showing a II of two $1+A-x$ chromosomes, and an unpaired chromosome 2. Note failure of the chiasma to terminalize in the $1+A$ chromosomes, even when these are paired as a II.

FIGURE 5.—Metaphase I in a dwarf, $2n+2A-2x$ plant, showing IV of two $1+A-x$ chromosomes and two chromosomes 2.

the greenhouse in October, where they produced new growth but remained slender and unthrifty. All nevertheless proved to be fertile. Meiotic configurations were analyzed in four of these plants. Two had a pair of $1+A$ chromosomes plus a normal chromosome 2 pair (fig. 5) and hence were tetrasomic for the A segment ($2n+2A$). The other two also had a pair of $1+A$ chromosomes, but only a single (normal) chromosome 2 (fig. 3, 4). They are therefore trisomic for the A segment, but monosomic for the centromere region of chromosome 2 ($2n+A-C$).

These plants provide the first instance so far obtained of transmission

through pollen of unbalanced gametes from the 2B-1 translocation. The $2n+2A$ plants can have been produced only by the functioning of $n+A$ pollen. It is most likely that the $2n+A-C$ plants were produced by $n+A$ sperm fertilizing $n-C$ eggs, since trisomics are often transmitted through pollen in cotton, while only one case of male transmission of a monosomic has been found (unpublished data).

The dwarf plants are the only phenotypically abnormal types so far recovered from 2B-1. Neither $2n-C$ nor $2n+A$ plants are dwarf. Tetrasomy sometimes produces abnormal phenotypes in cotton, but only the $2n+2A$ plants are tetrasomic (for the A segment). But both dwarf types are homozygous deficiencies for the hitherto neglected x segment of chromosome 1. It seems probable that the dwarf phenotype is due to the homozygous deficiency.

If this interpretation is correct it may enable us to determine whether the x segment was translocated to the broken end of C, or deleted. If the former, the homozygous translocation should be normal; if the latter, it should also be dwarf. Selfed progenies of the heterozygous translocation have not been studied since the realization that all of the cytological classes could be distinguished. It is probable, however, that at least one homozygous translocation was recovered in the 1949 progeny from 2B-1. This plant was characterized by a large bivalent with an unterminalized chiasma in the longer arm, like the 1 + A II shown in figure 4, and a second very small, attenuated bivalent, probably the C II. The plant in question was phenotypically normal, which makes it seem probable that the translocation was truly reciprocal. Further study of this point is not yet completed.[3]

TERMINALIZATION OF CHIASMATA

It has long been questioned whether the single terminal or nearly terminal chiasma per arm characteristic of metaphase bivalents in Gossypium represents a low original chiasma frequency, or well-advanced terminalization. Prophase stages are unfavorable for direct investigation of the question, and so far no three-point linkage groups have been available for an estimate of double crossover frequencies by genetic means.

Observations incidental to the present study suggest that the single interstitial chiasma seen proximal to A at metaphase represents the maximum number formed earlier.

At metaphase I in plants of the classes IV, $2n+A$ and $2n-C$, which are heterozygous for chromosome 1 + A, the interstitial chiasma proximal to A

[3] Since the manuscript was submitted for publication, four plants homozygous for the translocation have been identified cytologically. These four plants are phenotypically normal. In addition, from selfing the heterozygous translocation a plant has been recovered which shows chromosome configurations identical with those found in the $2n+2A-2x$ dwarfs (a pair of 1 + A chromosomes and a pair of normal chromosomes 2), except that it has in addition a C chromosome ($2n+2A+C-x$). This plant is also phenotypically normal. These findings support the conclusion that the translocation was reciprocal, and that the dwarf habit of $2n+2A-2x$ and $2n+A-C-2x$ plants is due to the homozygous deficiency for the x segment.

clearly fails to terminalize past the point of translocation. Even when the A arm is not paired with chromosome 2, a heteromorphic bivalent with one chiasma in each arm can often be identified by the long, single A arm projecting beyond the chiasma. In cells in which no chiasma is present proximal to A, the point of attachment is usually marked by a slight constriction or faintly stained region.

Failure of the chiasma to terminalize in heterozygotes for A is probably not due to mechanical difficulties inherent in the unequal length of the paired arms, or to change of homology (cf. DARLINGTON 1937, p. 510). Terminalization also fails in $2n + 2A$ and $2n + A - C$ plants, where both chromosomes 1 have A segments and are therefore of equal length and presumably homologous throughout. In a total of 90 pollen mother cells, the two A arms could be distinguished with certainty in 77 cells. The majority showed the unterminalized chiasma proximal to A. Those which lacked it showed the constriction at the point of attachment.

It appears, then, that the region of breakage and attachment of A in chromosome 1 serves to prevent pre-metaphase terminalization of chiasmata proximal

TABLE 3

Segregation of five characters in a selfed progeny of $2n - A$ plants from the cross recessive heterozygous translocation X dominant normal SL7-9.

Cytological type	Plant color		Leaf shape		Petals		Lint		Seed	
	Red	Green	Okra	Broad	Spot	No spot	Brown	White	Naked	Fuzzy
26 II	41	10	42	9	35	16	39	12	33	18
$2n - A$	27	11	25	13	31	7	28	10	22	16
Total	68	21	67	22	66	23	67	22	55	34

Expected 3 : 1 ratio = 66.75 : 22.25.

to it. (No evidence was seen that anaphase separation is obstructed, however). If this is true, it follows that not more than one chiasma is characteristic of the arm at prophase, since no more than one metaphase chiasma was ever seen proximal to A (in a total of about 575 cells from IV, $2n + A$, $2n - C$, $2n + 2A$ and $2n + A - C$ plants combined).

TESTS WITH GENETIC MARKERS

The transmission of an appreciable number of deficiency types $2n - A$ and $2n - C$, together with the $2n - 1$ plants recovered from $2n - C$, makes it possible to detect the presence or absence of marker genes on chromosome 2 by a modification of the monosomic method (CLAUSEN 1941; CLAUSEN and CAMERON 1944).

The three $2n - A$ plants from which the 1951 selfed progeny was grown were heterozygous for five dominant genes which entered the cross from the cytologically normal parent. If any of these five genes were located on the A segment, the selfed progeny should fail to segregate the recessive phenotype. The genetic data are shown in table 3. The progeny was segregating for all

five characters, with both 26 II and $2n-A$ classes including both dominant and recessive phenotypes for all five characters. We may therefore conclude that none of the loci marked in SL7-9 is located on the A segment.

The $2n-C$ plant from which the 1951 selfed progeny was grown was similarly heterozygous for SL7-9. Reference to figure 2 and table 2 will show that if one of the five genes were located on C, none of the F_2 plants should show the recessive phenotype, since the only segment homologous with C present in the parent was in the unbroken chromosome 2 from SL7-9.

Despite the rather small population, recessives were recovered in every cytological class for each character, except no petal spot in the $2n-C$ class, and all pairs of characters showed a good fit for a 3 : 1 ratio for the population as a whole (table 4). Therefore none of the five loci marked in SL7-9 is on the C segment of chromosome 2. Since C and A together should equal a whole chromosome 2, it follows that none of the markers is on any part of chromosome 2, a conclusion borne out by recovery of recessives for each gene pair in the $2n-1$ class.

TABLE 4

Segregation of five characters in a selfed progeny of a 2n – C plant from the cross recessive heterozygous translocation X dominant normal SL7-9.

Cytological type	Plant color		Leaf shape		Petals		Lint*		Seed	
	Red	Green	Okra	Broad	Spot	No spot	Brown	White	Naked	Fuzzy
$2n-C$	6	3	7	2	9	0	7	2	5	4
$2n-1$	6	1	4	3	5	2	5	2	5	2
$2n+A$	6	1	6	1	4	3	5	2	5	2
26 II	11	4	13	2	12	3	11	3	11	3
Total	29	9	30	8	30	8	28	9	26	11

Expected 3 : 1 ratio = 28.5 : 9.5.
*One 26 II plant not recorded.

It is now desirable to test whether any of the markers of SL7-9 is located on chromosome 1. It may be said at once that none is on the terminal (x) segment of chromosome 1, since one of the $2n+2A$ plants, a homozygous deficiency for the minute distal segment, nevertheless had all five dominant characters. No types deficient for other parts of chromosome 1 have been recovered from the translocation, and pairing relations make it unlikely that they can be recovered with ease. The possibility remains of determining whether any of the five genes in question is located on chromosome 1 close enough to the point of breakage to show linkage with the attached A segment from chromosome 2.

The heterozygous translocations recovered in 1950 were heterozygous also for the dominant markers carried by SL7-9 (red body, okra leaf, petal spot, brown lint, naked seed). These were backcrossed to a cytologically normal recessive line to produce the 1951 progeny. Ignoring the single plant which was monosomic for a third chromosome not involved in the translocation, 57 analyzed plants remain, distributed equally in four cytological classes (fig. 2). Of these four classes, two (IV and $2n+A$) are heterozygous for altered chro-

mosome 1 bearing the A segment, and two (26 II and $2n-A$) are homozygous for normal chromosome 1. These two combination cytological classes do not deviate significantly from a 1 : 1 ratio (table 5). As before, the dominant markers entered the cross with the normal chromosomes. A segregation of 1 : 1 is expected for each pair of markers in the backcross progeny, and none of the ratios differed significantly from the expectation.

Therefore, if none of the genes in question shows linkage with the point of translocation, the parental versus recombination ratio should also be 1 : 1. All of the linkage χ^2's are nonsignificant except that for the segregation of red and green (table 5). For the red-green pair, the χ^2 for parental versus recombination types is 5.07, P = .02–.05, which would seem to indicate that red and the point of translocation are not independent. But, as shown in table 5, the deviation is in the opposite direction from that expected if red-green were linked with the point of translocation; there is an excess of recombination rather than of parental types (20 parental : 37 recombination plants).

TABLE 5

Segregation of characters in a backcross progeny of a heterozygous translocation heterozygous for SL7-9.

Character pair	Parental classes			Recombination classes		
	IV & $2n+A$ recessive	26 II & $2n-A$ dominant	Total	IV & $2n+A$ dominant	26 II & $2n-A$ recessive	Total
Red vs. green*	9	11	20	15	22	37
Okra vs. broad	12	16	28	12	17	29
Petal spot vs. no petal spot	13	17	30	11	16	27
Brown vs. white	9	18	27	15	15	30
Naked vs. fuzzy	15	18	33	12	12	24

Expected parental vs. recombination: 28.5 : 28.5

	χ^2	P
*IV & $2n+A$ vs. 26 II & $2n-A$	1.421	.20–.30
Red vs. green	0.438	.50–.70
Parental vs. recombination	5.070	.02–.05
Total	6.969	.05–.10

If the significant χ^2 for linkage is assumed to indicate the location of the red locus on chromosome 1, the crossover percentage between red and the point of translocation is estimated to be 64.9 ± 6.3. Even with a sample of only 57 plants, it may be concluded that crossing over exceeded 50 percent, unless a 1 in 100 mischance in sampling occurred. It is planned to repeat the experiment with larger numbers to see if these puzzling results can be repeated, and if so, to obtain a more reliable estimate of the crossover frequency. While it has been shown that crossing over in excess of 50 percent can occur (FISHER and MATHER 1936; FISHER 1948), such cases appear to be rare. The cytological observations discussed above fail to suggest a meiotic basis for it in the present case, since they seem to indicate that only one chiasma is formed between A and the centromere of chromosome 1, and that the chiasma represents the usual single crossover between two of the four chromatids.

It may be mentioned in passing that the location of red on chromosome 1 is

not in accord with the evidence that the only anthocyanin locus (R_1) known to give red plant body in *G. hirsutum* is in the D genome (SILOW 1940, 1946; HARLAND and ATTECK 1941). The chromosome 1 pair forms one of the larger bivalents at metaphase I, the cytological inference being that it is a bivalent of the A genome.

DISCUSSION

In summary, a total of seven nondisjunctional complexes has been recovered from the 2B-1 translocation and its derivatives. One of these is a simple monosomic ($2n-1$). Three are in reality deficiency-duplication types, if the minute x segment is taken into account: $2n+\mathrm{A}\ (-\mathrm{x})$, $2n+2\mathrm{A}\ (-2\mathrm{x})$, and $2n+\mathrm{A}-\mathrm{C}\ (-2\mathrm{x})$. If the translocation is indeed reciprocal, as seems likely, the $2n-\mathrm{A}$ type must also actually be a deficiency-duplication ($2n-\mathrm{A}+\mathrm{x}$), while $2n+\mathrm{C}$ is actually a double duplication, $2n+\mathrm{C}+\mathrm{x}$, and $2n-\mathrm{C}$ is a double deficiency, $2n-\mathrm{C}-\mathrm{x}$. Both duplications and deficiencies for C, A and x are functional in the ovules, while duplications of A, and probably also of C and x, are pollen-viable. In addition, deficiencies of x are to some extent transmissible through the pollen. Since all seven genotypes are viable and fertile, it seems highly probable that several additional genotypes are recoverable by proper manipulation, if the need arises. In addition, there is a high degree of likelihood that the homozygous translocation may be established for use in further studies.

The recovery of $2n+2\mathrm{A}-2\mathrm{x}$ and $2n+\mathrm{A}-\mathrm{C}-2\mathrm{x}$ plants demonstrates for the first time in cotton, the pollen transmission of a small deficiency and its recovery in the homozygous condition. STEPHENS (1950), in a discussion of the possible role of cryptic structural hybridity in the differentiation of closely related species of Gossypium, points out that two assumptions are necessary to the theory: " (a) that small deficiencies would not always behave as pollen lethals and (b) that crossing-over would occur frequently between only partially homologous chromatids." The pollen transmission of deficiencies for x demonstrates that the first condition can exist in cotton. Moreover, this deficiency is of a size to render plausible its classification as a cryptic structural change, since its presence or absence cannot be detected except by inference at the meiotic stages which can be studied critically. In a formal sense, the pairing relations in the translocation and its derivatives, in particular the regular formation of the interstitial chiasma proximal to A, also satisfy the second condition, since a normal-appearing, two-chiasma bivalent can be formed even though the ends of the paired arms are not homologous. While estimation of the importance of cryptic structural hybridity between species must rest on other tests, these facts at least demonstrate that the cytological conditions for it can exist.

The occasional transmission of duplication gametes from translocations has been reported by a number of authors (e.g., BURNHAM 1934, 1948; LEWIS 1951; RHOADES 1933; SUTTON 1939). Recovery of deficiency-duplications has also been reported by BURNHAM (1932) in maize, SEARS (1939) in *Triticum vulgare*, and SMITH (1948) in *Triticum monococcum*. The deficiency-duplica-

tion described by SMITH is apparently analogous to the $2n + A - x$ class in the present study, though in his case the size difference between "A" and "x" is not so marked, both being rather short terminal segments.

So far as is known to the authors, however, no case has previously been reported in plants in which the array of unbalanced complexes approaches the theoretical possibilities as closely as in the present case. An explanation for the apparent rarity of such cases is not readily forthcoming. SMITH (1948) points out that deficiency-duplications ought to be fairly common in polyploid species, especially where deficiencies of whole chromosomes are known to be tolerated.

It may be pointed out that the extreme inequality of the interchanged arms, and the resulting effect on the location of chiasmata, render the present translocation unusually favorable for cytological detection of unbalanced complexes. It is not improbable that some such types have previously escaped detection. Because of its particularly convenient features, the 2B-1 translocation is the first to have been analyzed extensively in cotton. It is of course not certain or even probable that all translocations will yield the same array of unbalanced types. But it seems likely that if others can be found with the favorable features of 2B-1, some will prove to show similar behavior.

SMITH (1948) has discussed the possible uses of deficiency-duplication lines, and many other possibilities present themselves in special cases. The lines from 2B-1 are themselves useful. Perhaps more significant, however, are the implications they hold for future cytogenetic methods in cotton. The simplest models for studying linkage are those which make use of the monosomic method or its variants, since these are qualitative tests. While monosomics are now known to be transmissible in cotton in at least some cases, a promising method for their production has hitherto been lacking. Results of experiments with asynaptic plants have been disappointing. But translocations are rather easily induced by radiation, and the 2B-1 derivatives show that simple monosomics can be recovered from translocations. In practice, duplication-deficiency types may, however, prove both more easily recovered and more useful, since they are likely to be transmitted with greater regularity, and since some of them make it possible to determine concurrently the location of genes and the particular region of the chromosome concerned.

Aside from the possible contribution of 2B-1 and similar translocations to the cytogenetic exploration of *G. hirsutum* itself, certain of the derivatives present attractive possibilities of comparing the chromosomal and genetic anatomy of the allotetraploid species with that of their diploid relatives. Information of this sort should have considerable bearing on problems of transference of desirable characters from the diploid species into the cultivated allotetraploid cottons.

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