MEIOTIC CHROMOSOME BEHAVIOR IN SPECIES, SPECIES HYBRIDS, HAPLOIDS, AND INDUCED POLYPLOIDS OF GOSSYPIUM[1]

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INTRODUCTION

THE genus Gossypium, a member of the Malvaceae, includes the species of cultivated cottons. More than 100 species of Gossypium have been described, but recent workers include all the described types in fewer than 20 species (HUTCHINSON and GHOSE 1937; HARLAND 1939). The basic chromosome number in the genus is 13, and all species studied have either 13 or 26 pairs of chromosomes.

The cytological behavior of all species hybrids, except one, included in this paper has been reported previously by other workers. Further data on the meiotic chromosome behavior of species hybrids and haploids are given, together with a review of published data. Data on the meiotic chromosome behavior of several induced polyploids of Gossypium are given for the first time. In the present work emphasis is placed upon data relative to the amount of structural differences among chromosomes of different species and to the meiotic chromosome behavior of polyploids. These data are used in an attempt to define more accurately the relationships of Gossypium species and to make clearer the process of speciation of the genus.

GENERAL CONSIDERATIONS

The more comprehensive previous works on the meiotic chromosome behavior of Gossypium hybrids were published by SKOVSTED (1933, 1934, 1935a, 1937) and WEBBER (1935, 1939). These two authors adequately review the earlier papers on the subject. The data published by SKOVSTED, WEBBER, BARDUCCI and MADOO (1940), and ABRAHAM (1940) are summarized in table 1. In making the summary, it seemed desirable in some instances to combine data from two or more similar crosses. Some of the published cytological data which are unrelated to this study are omitted. In studying hybrids, SKOVSTED and WEBBER recorded the number of univalents, bivalents, trivalents, etc., and SKOVSTED recorded chiasma frequency. In some cases the data published independently by SKOVSTED and by WEBBER from the same or similar hybrids are greatly different.

[1] This work was initiated under the direction of the late PROF. E. M. EAST and submitted to HARVARD UNIVERSITY as part of a thesis in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Much of the cytological work was done while the author was Agent, U. S. DEPARTMENT OF AGRICULTURE, Bureau of Plant Industry, Raleigh, N. C.

SKOVSTED (1937) separated Gossypium species into three, WEBBER (1939) separated them into four, and HARLAND (1939) separated them into six general types. These authors considered morphology and chromosome number and gave some attention to chromosome pairing in hybrids and to geographical distribution. In the present work, with more emphasis placed on chromosome pairing and structural differences among the chromosomes, the species are divided into six types. One of HARLAND'S types is divided, while two others are combined.

In table 2 and throughout this paper formulae are used to describe the chromosome composition of species and hybrids. These formulae are written by using different capital letters to designate structurally unlike sets of chromosomes and by placing numbers before the letters to indicate the number of times sets are present (BEASLEY 1940a). Closely related sets are distinguished by placing subscripts 1, 2, etc., after the letters that designate the sets. Sets in natural polyploids are enclosed with parentheses with subscripts after the last parenthesis. The evidence for the relationships indicated among the sets of chromosomes in table 2 is given later in this paper, but the formulae are used throughout because they facilitate the description and designation of plants.

Since emphasis in this paper is placed on structural differences among chromosomes of different species, particular attention is given to chromosome bridges at first meiotic anaphase. Such chromosome bridges have been shown in many plants to result from crossing over in individuals heterozygous for inversions or inverted translocations which gives chromosomes with two centromeres. The bridges are formed when the centromeres go to opposite poles.

The concept that chromosome bridges result from differences in chromosome structure was advanced by MCCLINTOCK (1933). She found that crossing over sometimes occurs in heterozygous inversions in which homologous parts pair by forming a loop. This crossing over gives dicentric chromosomes which frequently form bridges at anaphase. MÜNTZING (1934) pointed out that chromosome bridges in species hybrids are the result of structural differences in chromosomes. His diagrams I to IV illustrate how bridges can arise from certain types of translocations. RICHARDSON (1936) diagrammed several ways in which bridges may arise in structural hybrids. SAX (1937) reported chromosome bridges in Paeonia to result from crossing over in heterozygous inversions. He found inversions in three of the five chromosomes and reported that heterozygous inversions caused partial asynapsis. Numerous other workers, including DARLINGTON (1936, 1937), DOBZHANSKY (1937), UPCOTT (1937), WOODS (1937), EMSWELLER and JONES (1938), STEBBINS and ELLERTON (1939), and SWANSON (1940), and several others, have studied structural differences

in chromosomes. Species with large chromosomes were usually used. The number of structural differences was one to five, and bridges could be interpreted readily as resulting from heterozygous inversions or inverted translocations.

In the present work some first meiotic anaphase figures of species hybrids were found to have a larger number of bridges than is usually reported. It is believed that comparable numbers have been observed in other genera. It has been suggested that the large number of attenuated chromosomes in certain hybrids could be a physiological effect resulting from the hybridization of the species. This is untrue in Gossypium, for the chromosomes fail to become attenuated in polyploids produced from the hybrids. The drawings of meiotic figures of Nicotiana hybrids published by GOODSPEED (1934) indicate that types with a reduced and variable amount of pairing frequently give chromosome bridges at anaphase.

In the work with Gossypium, or other species with small chromosomes, it is hardly possible to make the detailed observations that can be made on material with large chromosomes. Perhaps one reason figures interpreted in the present work as bridges were not so interpreted by previous workers is that the number of fragments is usually less than the number of bridges. It is possible, however, that the fragments are rarely freed at anaphase. MCCLINTOCK (1938) found that in about one-third of her observations a fragment was "associated at one of its ends with the end of one of the normal chromatids." Probably another reason why the number of fragments is lower than the number of bridges is that in many cases the bridges are not the result of crossing over in inversions, but the result of changes in homology which prevent terminalization (DARLINGTON 1937). The point emphasized in this paper is that bridges, whether they arise from crossing over in inversions or by other means, result from structural differences among the chromosomes of different species. Structural differences are interpreted here to include segments with genes that may not be in one of the two species in a hybrid—that is, non-homologous segments.

It is customary for conclusions as to relationships between species to be based largely upon the amount of chromosome pairing in hybrids between the species; it is known, however, that changes in chromosome structure, which influence chromosome pairing, apparently can occur without accompanying genetic effect (ANDERSON 1935; STEBBINS 1938). In Gossypium it is probable that the amount of chromosome pairing is a good measure of relationship, for, in general, it agrees with geographical distribution and external morphology.

TECHNIQUE

Flower buds were killed in a mixture of 30 parts acetic acid and 70 parts

TABLE 1

*Meiotic chromosome behavior in species hybrids of Gossypium.**

GROUP	TYPES CROSSED	SPECIES CROSSED†	BEHAVIOR OF CHROMOSOMES AT M I AND A I								AUTHOR
			I‡	II	III	IV	V	VI	CHIASMA FREQUENCY PER BIVALENT	EVIDENCE OF STRUCTURAL DIF. IN CHROMOSOMES	
1	2A×2A (Asiatic 13×Asiatic 13)	*sanguineum*×*africanum*	sometimes 2	13.0							WEBBER 1935
		herbaceum×*cernuum*	0.2	12.9							WEBBER 1939
		herbaceum×*arboreum*	sometimes 2	13.0					reduced from normal		SKOVSTED 1933
2	2A×2B (Asiatic 13×African 13)	*arboreum*×*anomalum*	3.1	10.5	0.2	0.4			1.37	Text fig. 3 gives evidence of A I bridges	SKOVSTED 1937
		arboreum var. *nanking*×*anomalum*	1.7	11.7	0.2	0.1			1.48		SKOVSTED 1937
		herbaceum var. *africanum*×*anomalum*	2.5	11.8					1.41	Text fig. 4 gives evidence of A I bridges	SKOVSTED 1937
		sanguineum×*anomalum*	2.4	11.8							WEBBER 1939
3	2A×2C (Asiatic 13×Australian 13)	*arboreum*×*sturtii*	2.8	9.8	0.6	0.4			1.32	Text figs. 7 & 8 give evidence of A I bridges	SKOVSTED 1937
		herbaceum×*sturtii*	21.6	2.2							WEBBER 1939
4	2A×2D (Asiatic 13×American 13)	*arboreum*×*thurberi*	9.6	7.8	0.2	0.05			1.16	Text figs. 12–15 show bridges	SKOVSTED 1937
		nanking×*thurberi*	24.0	1.0							WEBBER 1939
5	2A×2E (Asiatic 13×Arabia-India 13)	*arboreum*×*stocksii*	12.0	7.0							ABRAHAM 1940
		herbaceum var. *wightiana*×*stocksii*	19.6	3.2					1.05		SKOVSTED 1937
		arboreum var. *nanking*×*stocksii*	17.3	4.4					1.07		SKOVSTED 1937
		arboreum×*stocksii*	13.5	6.2					1.11	Text fig. 6 shows bridges	SKOVSTED 1937
		sanguineum×*stocksii*	24.1	0.9							WEBBER 1939
6	2A×2(AD) (Asiatic 13×American 26)	*arboreum*×*barbadense*	14.4	8.4	1.0	0.6	0.3	0.1		Text figs. 6–8 show evidence of bridges	SKOVSTED 1934
		asiatic×*hirsutum*	13.7	11.9		0.35					WEBBER 1935
		cernuum×*hirsutum*	13.2	12.7	0.1					Rarely nondisjunction occurred	WEBBER 1939
7	2B×2D (African 13×American 13)	*anomalum*×*aridum*	21.2	2.4	0.05				1.03		SKOVSTED 1937
		anomalum×*thurberi*	14.3	5.7	0.01				1.12	Text fig. 11 shows bridges	SKOVSTED 1937
		anomalum×*davidsonii*	19.9	3.1					1.08	Text fig. 10 shows 1 bridge	SKOVSTED 1937
		anomalum×*davidsonii*	25.6	0.2							WEBBER 1939
8	2B×2(AD) (African 13×American 26)	*anomalum*×*barbadense*	33.8	2.6					1.05	Text fig. 22 shows 4 bridges	SKOVSTED 1937
		anomalum×(*barbadense*×*schottii*)	18.1	10.3	0.1						WEBBER 1939
		anomalum×(*contextum*×*hopi*)	17.8	10.5	0.1						WEBBER 1939
9	2C×2D (Australian 13×American 13)	*sturtii*×*harknessii*	24.6	0.7						Reports fragmenting chromosomes	WEBBER 1935
		sturtii×*thurberi*	24.5	0.7						Fig. 11 shows 5 bridges	WEBBER 1935, 1939
		sturtii×*armourianum*	24.1	1.0						Reports fragmenting chromosomes	WEBBER 1935

		sturtii×*armourianum*	8.5	8.2	0.3	0.1			Skovsted 1937
		sturtii×*davidsonii*	14.9	5.3	0.1	0.03	1.06	Text figs. 6–8 show several bridges	Skovsted 1935
		sturtii×*davidsonii*	24.0	1.0				Reports fragmenting chromosomes	Webber 1935
10	2C×2(AD) (Australian 13×American 26)	*sturtii*×*barbadense*	37.2	0.9				Reports fragmenting chromosomes	Webber 1935
		sturtii×*barbadense*	32.5	3.1	0.1		1.0	Text fig. 25 shows 5 bridges	Skovsted 1937
		sturtii×*hirsutum*	21.0	7.6	1.0				Skovsted 1937
		sturtii×*hirsutum*	37.9	0.5					Webber 1939
		sturtii×*purpurascens*	24.3	6.0	0.8	0.05	1.05	Text fig. 26 shows 8–10 bridges	Skovsted 1937
		sturtii×*darwinii*	27.1	5.3	0.4	0.05	1.02	Text fig. 27 shows 4–6 bridges	Skovsted 1937
11	2D×2D (American 13×American 13)	*thurberi*×*aridum*	sometimes 2	13.0			1.73		Skovsted 1937
		amourinaum×*thurberi*	sometimes 2	13.0			1.57	Text fig. 2 shows 1 probable bridge	Skovsted 1937
		amourianum×*thurberi*	0.6	12.7					Webber 1939
		armourianum×*aridum*		13.0					Skovsted 1937
		davidsonii×*klotzschianum*	0.1	12.95					Webber 1939
		harknessii×*armourianum*		13.0					Webber 1935
		harknessii×*thurberi*	0.8	12.6					Webber 1939
12	2D×2AD (American 13×American 26)	*armourianum*×*hirsutum*	13.0	13.0					Webber 1934, 1935
		armourianum×*hirsutum* (Vy)	13.5	12.2	0.4				Skovsted 1937
		armourianum×*hirsutum* (V_4)	11.8	13.4	0.2				Skovsted 1937
		thurberi×*hirsutum*	13.1	12.7	0.2				Webber 1939
		thurberi×*hirsutum*	13.5	12.5	0.2		1.54		Skovsted 1937
		harknessii×*barbadense*	13.0	13.0					Webber 1935
		armourianum×*contextum*	13.0	12.7		0.1			Webber 1935
		aridum×*barbadense*	14.0	12.3	0.2		1.73	Text fig. 16 shows possible bridges	Skovsted 1937
		aridum×*hirsutum*	13.2	12.4	0.4			Text fig. 17 shows 1 bridge	Skovsted 1937
		aridum×*purpurascens*	13.0	12.0	0.6	0.05	1.64		Skovsted 1937
		armourianum×*purpurascens*	13.8	12.4	0.04		1.49	Text fig. 19 shows possible bridges	Skovsted 1937
		armourianum×*tailense*	14.5	12.1	0.1		1.55		Skovsted 1937
		armourianum×*barbadense*	14.8	12.0	0.1				Skovsted 1937
		armourianum×*darwinii*	13.2	12.0	0.6				Skovsted 1937
		thurberi×*barbadense*	12.7	12.4	0.5			Text fig. 21 shows 2 or more bridges	Skovsted 1937
		thurberi×*tailense*	13.4	12.1	0.5				Skovsted 1937
		hirsutum×*raimondii*	12.6	11.6	0.9	0.1			Barducci and Madoo 1940
13	2E×2(AD) (Arabia-India 13×American 26)	*barbadense*×*stocksii*	37.9	0.6			1.00		Skovsted 1937

* Although the species names given by the authors fail in some cases to conform to the classification given by Hutchinson and Ghose (1937) and Harland (1939), the names have been retained here, for without specimens it is hardly possible to change the names to follow the recent classifications.

† In these species crosses the first name does not necessarily indicate that it was used as female.

‡ I—univalent, II—bivalent, etc.

TABLE 2

Types of Gossypium species as determined by their geographical distribution, morphology, chromosome pairing, chromosome number, and structural differences among sets of chromosomes.

	CHROMOSOME CONSTITUTION
Asiatic 13-chromosome	
G. herbaceum L.	$2A_1$
G. arboreum L.	$2A_2$
African 13-chromosome	
G. anomalum Wawra. and Peyr.	$2B_1$
Australian 13-chromosome	
G. sturtii F. Muell.	$2C_1$
American 13-chromosome	
G. thurberi Tod.	$2D_1$
G. armourianum Kearney	$2D_2$
G. harknessii T. S. Brandeg.	$2D_2$
G. davidsonii Kellogg	$2D_3$
G. klotzschianum Anderss.	$2D_3$
G. aridum (Rose and Standley) Skovsted	$2D_4$
G. raimondii	$2D_5$
Arabia-India 13-chromosome	
G. stocksii M. Mast	$2E_1$
American 26-chromosome	
G. hirsutum L.	$2(AD)_1$
G. barbadense L.	$2(AD)_2$

of either 95 percent or absolute ethyl alcohol. It was found that under average growing conditions, meiotic metaphase figures were common in material collected from 7 to 8 a.m., and anaphase figures were frequent in material collected one hour later. Unusually warm nights followed by sunny days advance the stages somewhat, while cool, cloudy weather retards the stages. Better slides were obtained after buds were left in acetic-alcohol at least three days. The stain used was BELLING'S (1926) iron-aceto-carmine. Most slides were sealed with a mixture of equal parts of gum mastic and paraffin; however, slides sealed in this manner usually spoiled after a few weeks. It was found that a self-sealing aceto-carmine mixture recommended by ZIRKLE (1937) caused too much plasmylosis. Some unsealed slides were made permanent by removing the cover glasses in 70 percent alcohol and taking the slides and cover glasses through the remainder of the alcohol series to xylol and then mounting in balsam. In making sealed slides permanent, the method of MCCLINTOCK (1929) was followed.

CHROMOSOME BEHAVIOR IN PURE SPECIES

SKOVSTED (1933) found no abnormalities at first meiotic metaphase (M I) in PMC of *Gossypium arboreum*, $2A_1$, a 13-chromosome cultivated Asiatic cotton. In a triploid Asiatic cotton, however, he found that most

of the M I plates showed one or more associations of more than three chromosomes. In a later paper (1935a), he reported autosyndesis in both sets of chromosomes in a cross between two distantly related 13-chromosome species. WEBBER (1934) reported 11 percent of first anaphase (A I) figures of an Asiatic 13-chromosome species to have lagging bivalents, and 9.5 percent of second meiotic metaphase (M II) plates to have less than the haploid number of chromosomes. In the present work, although the studies made were not extensive, no abnormalities were found in normal 13-chromosome species. SKOVSTED (1933, 1937) reported chiasma frequency in Gossypium to average about 1.75 per bivalent in pure species.

A normal M I of a PMC of *G. arboreum* var. *neglectum*, $2A_2$, is shown in Plate 1, figure A, and one of *G. hirsutum*, $2(AD)_1$, is shown in Plate 1, figure B. Sharp disjunction of the chromosomes, a characteristic of pure Gossypium species, may be seen in the A I figure of *G. hirsutum* shown in Plate 1, figure C.

Meiosis in 26-chromosome Gossypium species frequently shows abnormalities. WEBBER (1934) reported 7.6 percent of M I figures to have associations of four chromosomes, and later (1938) found 14 percent of the pollen mother cells at M I to have either univalents, associations of four or both. The maximum number of univalents or of associations of four chromosomes found in a single cell was two. In the present work normal 26-chromosome species were studied to a limited extent. Bridges, lagging chromosomes, and groups of four chromosomes occasionally were found.

CHROMOSOME BEHAVIOR IN AUTOPOLYPLOIDS

In M I of 14 PMC of a $4A_1$ plant from *G. herbaceum* the number of quadrivalents ranged from 7 to 12 and averaged almost 10. In some cases it was difficult to determine whether there were two or four chromosomes in a group, but the total number of chromosome groups was counted and the number of quadrivalents calculated. Each of two cells had one univalent and one trivalent. A count of 25 A I figures gave 11 that separated 26-26, eight that separated 25-27, two that separated 24-28, one that separated 23-29, and three had chromosomes in the cytoplasm that probably would not have reached either pole.

Similar results at A I were secured in tetraploid Datura (BELLING and BLAKESLEE 1924) and in tetraploid *Lycopersicum esculentum* (Upcott 1935). JØRGENSEN (1928) and HUMPHREY (1934) reported abnormalities to be rare at A I of tetraploid *L. esculentum*.

In order to calculate the number of A I figures that had balanced 26 chromosome sets, it is necessary to subtract two, the number of 24-28 separations that were found, from the 26-26 separations, since if two quadrivalents separate 1-3, there is, so far as is known, an even chance

that the unbalance will be in opposite directions, which would give a count of 26-26 at A I. This makes it necessary to double the number in the 24-28 class (BELLING and BLAKESLEE 1924). These corrections indicate that nine of the 25 separations should have been balanced 26-26 distributions, and the plant should have given 36 percent of viable pollen. Some flowers have as much as 30 percent of apparently viable pollen.

From the data on the $4A_1$ Gossypium it was calculated that quadrivalents separate in a 3-1 manner about 13 percent of the time (22 cells averaged ten quadrivalents each, 28 quadrivalents separated 3-1). If it is assumed that there is an even chance of quadrivalents separating 2-2 or 1-3, the chance of ten quadrivalents in the same cell giving a balanced type would be $(\frac{1}{2})^{10}$ or 1/1024. The chance of the separations being something other than 26-26 would be about 4 to 1. Considering only the cells in which all the chromosomes were going to the poles, the 26-26 separations in the $4A_1$ Gossypium amounted to 50 per cent. It appears that quadrivalents separate 3-1 more than 13 percent and less than 50 percent of the time. BELLING and BLAKESLEE (1924) calculated that quadrivalents in tetraploids of Datura separate 3-1 about three percent of the time.

It was observed that bridges are sometimes present at A I in $4A_1$ plants. The most logical explanation is that some chromosomes within the basic A set have segments in common, and if segments fail to pair with like segments in homologous chromosomes, they will sometimes pair with like

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EXPLANATION OF PLATE I

FIGURE A.—First meiotic metaphase of an Asiatic 13-chromosome species *G. arboreum* var. *neglectum*.

FIGURE B.—First meiotic metaphase of an American 26-chromosome species *G. hirsutum*.

FIGURE C.—First meiotic anaphase of American 26-chromosome species *G. hirsutum* showing sharp separation of chromosomes.

FIGURE D.—First meiotic anaphase of haploid, $(AD)_1$, plant of *G. barbadense* showing 26 chromosomes.

FIGURE E.—First meiotic metaphase of octoploid $2[(AD)_1(AD)_2]$ from *G. hirsutum* and *G. barbadense*. Most of the chromosomes are paired as quadrivalents.

FIGURE F.—First metaphase of American 26- ×American 13-chromosome species, *G. hirsutum* ×*G. harknessii*, $(AD)_1D_2$, showing 12 associations of two chromosomes and 15 univalents.

FIGURE G.—First meiotic metaphase showing most of the chromosomes paired in a hybrid $A_2D_1(AD)_1$ produced by crossing an amphidiploid synthesized from an Asiatic 13- and an American 13-chromosome species with an American 26-chromosome species (*G. arboreum*×*G. thurberi*, amphidiploid)×*G. hirsutum*.

FIGURE H.—First meiotic anaphase of American 26- ×Australian 13-chromosome species, *G. hirsutum*×*G. sturtii*, $(AD)_1C_1$, showing eight bridges.

FIGURE I.—First meiotic anaphase of hexaploid $2[(AD)_1C_1]$ showing most of the chromosomes separating without forming bridges.

FIGURE J.—First meiotic anaphase of American 26- ×African 13-chromosome, *G. barbadense* ×*G. anomalum* $(AD)_2B_1$ showing nine or ten bridges.

FIGURE K.—First meiotic metaphase with 39 bivalents of a hexaploid from American 26- ×African 13-chromosome $2[(AD)_1B_1]$.

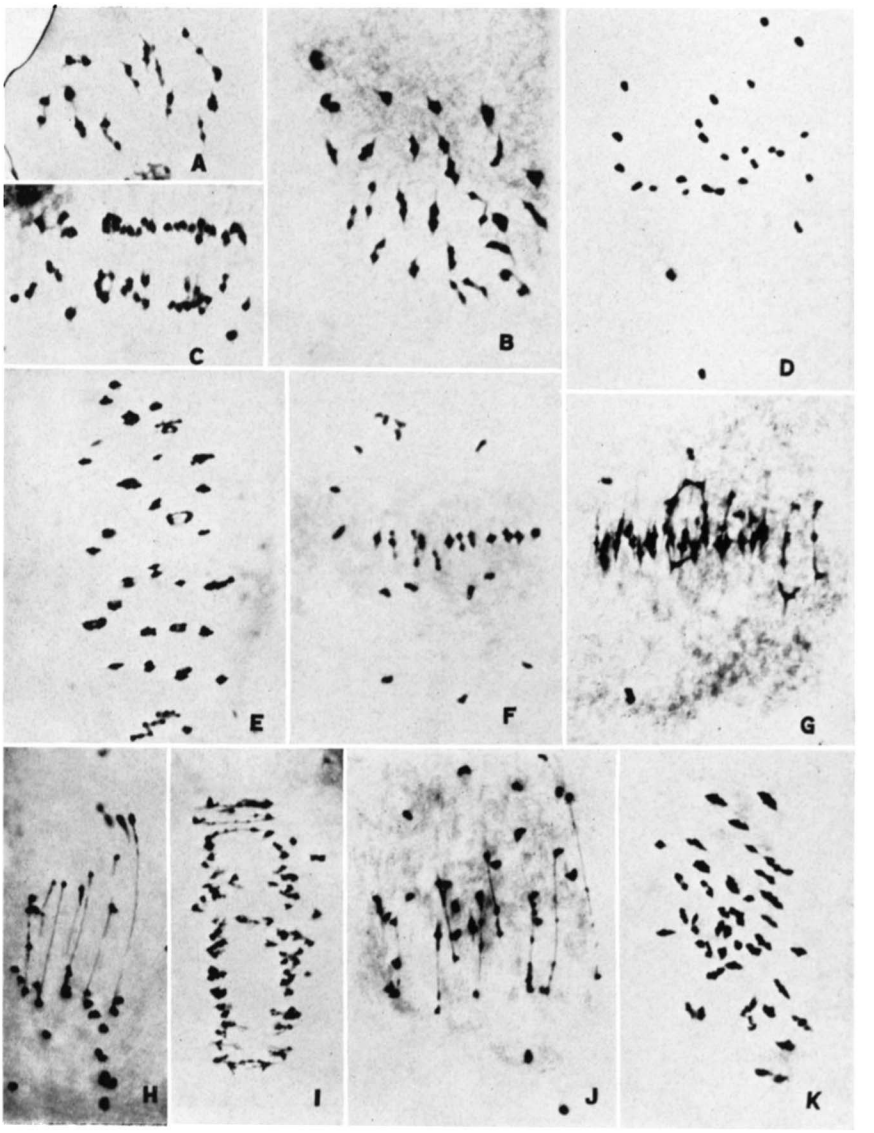

PLATE 1

segments in otherwise non-homologous chromosomes. The autosyndesis reported by SKOVSTED (1935a) and the presence of associations of more than three chromosomes in a triploid (SKOVSTED 1933) also indicate that this is true.

Doubling the chromosome number in *G. hirsutum*, $2(AD)_1$, and *G. barbadense*, $2(AD)_2$, gave $4(AD)_1$ and $4(AD)_2$ plants. At M I of these plants it usually was impossible to determine definitely whether a few associations of chromosomes were bivalents or quadrivalents (Plate 1, fig. E). It was possible, however, to count the number of chromosome bodies in some M I figures, and univalents could be identified. With the number of univalents identified and the total number of chromosome associations of two or more known, the number of quadrivalents was calculated. It was assumed that there was a trivalent for each univalent, and this was found to be true in a number of figures.

At M I 33 cells of a $4(AD)_1$ plant averaged 17.5 quadrivalents, 0.4 trivalents, 16.2 bivalents, and 0.4 univalents, while 18 cells of a $4(AD)_2$ plant averaged 18.1 quadrivalents, 0.8 trivalents, 14.2 bivalents, and 0.8 univalents. MENDES (1940) reported most of the A I and A II figures of an octoploid of *G. hirsutum* to have 52 chromosomes at each pole. He gave no data on M I. The high percentage of quadrivalents and the frequent presence of univalents and trivalents found in octoploids in the present work indicate that most of the microspores and megaspores of such plants would be unbalanced.

CHROMOSOME BEHAVIOR IN HAPLOIDS

Haploid, (AD), plants from American tetraploid species of Gossypium were reported by SKOVSTED (1933) to have a small number of chromosome associations at M I. WEBBER (1938) reported an average of 0.2 associations of two chromosomes and occasional fragmenting chromosomes. In the present work, both $(AD)_2$ (haploids of *G. barbadense*) and $(AD)_1$ (haploids of *G. hirsutum*) were studied cytologically. The interpretations of figures at M I and A I of $(AD)_2$ plants were usually subject to doubt, for frequently two chromosomes were together, as shown in Plate 1, figure D, and it was impossible to tell whether they were joined by chiasmata. If figures of that type are considered as associations of two chromosomes, an average of 0.8 bivalents was found at M I in $(AD)_2$ plants.

More conclusive data were secured from the A I figures of $(AD)_1$ plants of *G. hirsutum*, since associations of two chromosomes usually give bridges at A I (fig. 1). The average number of chromosome bridges at A I in 66 cells was 1.7, and the maximum number was five. The difference in the number of chromosome associations in $(AD)_1$ and $(AD)_2$ plants is probably genic, rather than chromosomal, for there are slight, if any, structural differences evident in crosses between $2(AD)_1$ and $2(AD)_2$ species.

FIGURE 1.—First meiotic anaphase of haploid $(AD)_1$ plant of *G. hirsutum* showing four bridges.

FIGURE 2.—First meiotic metaphase of hexaploid $2[(AD)_1A_1]$ from American 26- × Asiatic 13-chromosome species, *G. hirsutum*×*G. herbaceum*, showing univalents and multivalents.

FIGURE 3.—First meiotic anaphase of hexaploid $2[(AD)_1A_1]$ showing an extra chromosome (fragment?) at each pole.

FIGURE 4.—First meiotic anaphase of hexaploid $2[(AD)_1A_1]$ showing aberrant chromosome behavior.

FIGURE 5.—First meiotic metaphase of tetraploid $2A_1(AD)_1$ from *G. herbaceum* autotetraploid ×*G. hirsutum* showing the majority of the 3A sets associated as trivalents.

Associations of two chromosomes are common in haploids derived from polyploids, and in some cases they are present in haploids from diploids (DARLINGTON 1937, IVANOV 1938). An average of 1.7 and a maximum of five chromosome bridges at A I of $(AD)_1$ plants show that there is considerable duplication of chromosome segments in 2(AD) species. In fact, $(AD)_1$ plants behave cytologically much like the hybrid B_1D_4 of *G. anomalum*×*G. aridum* (table 1, group 7).

CHROMOSOME BEHAVIOR IN HYBRIDS AND POLYPLOIDS OF CLOSELY RELATED SPECIES

The hybrid A_2A_1 of *G. arboreum* var. *typicum*×*G. herbaceum* var. *africanum* frequently showed a bridge and fragment at A I or A II, and figures were found with one association of four chromosomes. Sometimes there was evidence of two bridges, and one A I was found with an extra chromosome going to one pole. Figures that appeared normal were common. SKOVSTED (1933) reported that a cross of *G. herbaceum*×*G. arboreum* had a lower chiasma frequency than the pure species and that sometimes the chromosomes separated 12-14 at A I. From a study of similar crosses, WEBBER (1935) reported an occasional lagging bivalent and some M II plates with 12 and 14 chromosomes. The presence of a bridge accompanied by a fragment and M I figures showing one association of four chromosomes is good evidence that the chromosomes of these two species differ by at least one translocation. WEBBER (1935) found no irregularities in a cross of two $2D_2$ species, *G. harknessii*×*G. armourianum*. SKOVSTED (1937) reported very few irregularities in three crosses between American 13-chromosome species. The cross D_2D_1, *G. armourianum*×*G. thurberi*, had a reduced chiasma frequency, and SKOVSTED's text figure 2 shows a probable bridge. One cell in his cross *G. thurberi*×*G. aridum*, D_1D_4, had 12 bivalents and two univalents. The data available (table 1, group 11) indicate that American 13-chromosome species have a slight amount of structural differentiation among their chromosomes.

A number of hybrids between 26-chromosome American, 2(AD), species were studied by WEBBER (1935, 1939). He reported that there are usually 26 bivalents at M I, but sometimes one, two, or three associations of four chromosomes were present, and the chiasma frequency was apparently somewhat reduced. Some of these crosses were studied in the present work, and the results indicate that chromosome irregularities occur more frequently in crosses of *G. hirsutum*×*G. barbadense* and *G. hopi* Lewton×*G. hirsutum* than in pure species.

Octoploids, $2[(AD)_1(AD)_2]$, produced by doubling the chromosome number in F_1 hybrids of *G. hirsutum*, $2(AD)_1$,×*G. barbadense*, $2(AD)_2$, had more than half the chromosomes associated in quadrivalents at M I (Plate

1, fig. E). The average number of quadrivalents in 24 cells was 15.2 or 58 percent of the maximum number. The number of quadrivalents per cell varied from 4 to 23. There was an average of 0.7 trivalents and 0.7 univalents per cell. The average of 15.2 quadrivalents per cell is just slightly lower than the average of 17.5 quadrivalents found in an octoploid of *G. hirsutum*, and the average of 18.1 found in an octoploid of *G. barbadense*. This shows that there is little preferential pairing of the chromosomes in the octoploid from F_1 hybrids of the two species, and it is further evidence that there is little structural differentiation of the chromosomes of *G. hirsutum* and *G. barbadense*.

CHROMOSOME BEHAVIOR IN HYBRIDS AND POLYPLOIDS OF DISTANTLY RELATED SPECIES

Three species of Gossypium—*stocksii*, 2E, of Arabia-India, *sturtii*, 2C, of Australia, and *anomalum*, 2B, of Africa—seem to be distantly related to each other and to all other species. These three species, together with the three groups of species (Asiatic, 2A; American 13-chromosome, 2D; and American 26-chromosome, 2(AD), give six distantly related types of Gossypium (table 2).

American 26- ×Asiatic 13-Chromosome Species

Hybrids of American 26-chromosome, 2(AD), and Asiatic 13-chromosome, 2A, cottons were studied by SKOVSTED (1934), FENG (1935), and WEBBER (1935). These authors usually found 13 to 15 univalents and 12 to 13 bivalents. SKOVSTED's results differed from the others in that he found higher associations to be common (table 1, group 6). Some of SKOVSTED's figures show evidence of bridges. The data collected on this cross in the present work (table 3), though similar to that collected by SKOVSTED, differ in that figures were found with fewer than 13 univalents and the average number of univalents is lower. Most of the present studies were based on crosses of *G. hirsutum*×*G. herbaceum*, although some data were collected on a cross of *G. barbadense*×*G. herbaceum* and one of *G. hirsutum* ×*G. arboreum*. The chromosome behavior in the three crosses differed slightly in number and types of multivalent associations.

Two associations of four chromosomes were common at M I (table 3). At A I the chromosomes sometimes separate without definitely showing bridges, but one to three bridges were common. It is believed that most of the bridges involve chromosomes from the two parent species, since in American 26-chromosome species the duplicated segments within sets, which were shown from the study of haploids to be present, are small in comparison to the segments duplicated in the nearly homologous sets from the different species.

The frequent presence of 13 univalents, 11 bivalents, and two associations of four chromosomes at M I, and of one to three bridges at A I, show that two of the three sets of 13-chromosomes in triploid (AD)A hybrids are similar, but that they differ by at least two translocations and perhaps one or more inversions.

TABLE 3

Chromosome behavior at M I of crosses between American 26- and Asiatic 13-chromosome species.

COMBINATION NUMBER	Number of						FRAGMENTS	*G. hirsutum* (NEW BOYKIN) × *G. herbaceum*	*G. hirsutum* (RED LEAF) × *G. herbaceum*	*G. barbadense* (PIMA) × *G. herbaceum*	TOTAL
	I*	II	III	IV	V	VI					
1	13	13					1		2		2
2	13	13							1	1	2
3	15	12								1	1
4	13	11		1				3	1		4
5	13	11		1			1	1		1	2
6	12	11			1			1			1
7	15	10		1					1		1
8	11	10		2					1		1
9	13	9		2				8	5	4	17
10	13	9		2			1		1		1
11	12	9	1			1				1	1
12	17	9		1					1		1
13	12	8	1	2				1			1
14	11	8	1	1	1				1		1
Total	469	357	3	49	2	1	3	14	14	8	36
Average	13.03	9.92	0.1	1.36	0.05	0.03					

* I—univalent; II—bivalent, etc.

SKOVSTED (1934) emphasized that 26-chromosome species have a set of 13 chromosomes that tend to be larger than the other set of 13. He also noted that it is the larger set from the 26-chromosome species that pairs in the triploid with the set from the 13-chromosome Asiatic species, which corresponds to the larger set in size. This observation of SKOVSTED'S was confirmed in the present work.

The chromosome number was doubled in triploids of *G. hirsutum* × *G. herbaceum* to give fertile hexaploids, $2[(AD)_1A_1]$. At M I of these hexaploids some of the chromosomes usually are in complex associations. Figures frequently had one or more univalents, association of three or of four chromosomes, or a combination of the three (fig. 2). Associations of more than four chromosomes were seen.

Since anaphase separation is more important in determining the viability of gametes than M I association, and also tends to reflect the results of M I associations, three A I figures were analyzed to get a more accurate interpretation of chromosome behavior in this hexaploid. In one figure the separation was 40-38, with evidence of one fragment. One anaphase (fig. 3) had 40 chromosome bodies going to each pole. A third A I figure (fig. 4) had several fragments and evidence of bridges. At A I lagging chromosomes, bridges, and fragments are common, but the number of these in a given anaphase is usually only one or two.

The M I and A I behavior indicate that almost every megaspore and microspore is unbalanced. This is expected, for M I and A I chromosome behavior in the triploids shows that two of the three sets of chromosomes are closely related. The chromosome behavior of the hexaploid fits the hypothesis as indicated in the formula $2[(AD)_1A_1]$ of four A sets of chromosomes. On the other hand, the amount of chromosome differentiation evidenced in the triploids has considerable influence on chromosome pairing, for the number of quadrivalents is much less than would be expected if four identical sets were present. Associations of more than four chromosomes indicate that chromosomes not structurally alike will sometimes pair even though completely homologous chromosomes are present; this is known, however, from pairing in natural species.

A hybrid pertinent to the clarification of chromosome relationship of cultivated American 26- and Asiatic 13-chromosome cottons was studied by SKOVSTED (1934). This hybrid had 52 chromosomes, and SKOVSTED had evidence that the hybrid resulted from the fertilization of a diploid Asiatic egg with a reduced gamete from 26-chromosome cotton. At M I this hybrid behaved as if it had three like sets and one differentiated set of chromosomes, for it averaged 15.8 univalents, 5.9 bivalents, 6 trivalents, 1 association of four, 0.4 association of five, and 0.1 association of six chromosomes. This is pertinent evidence that one 13-chromosome set in American 26-chromosome cotton is, in a general way, homologous with the 13-chromosome set in Asiatic cottons.

Plants with essentially the same chromosome composition, $2A_1(AD)_1$, were produced during the present study by pollinating stigmas of an autotetraploid Asiatic plant with pollen of a 26-chromosome species. Studies of these plants have confirmed SKOVSTED's conclusions. The drawing shown in figure 5 is similar to his drawings.

American 26- ×American 13-Chromosome Species

Hybrids of $2(AD)\times 2D$, 26- ×13- chromosome American species, show a meiotic chromosome behavior similar to that described for $2(AD)\times 2A_2$, American 26- ×Asiatic 13-chromosome species. There are important dif-

ferences, however, in the two types of hybrids. In the cross American 26- ×American 13-chromosome cottons the set of small chromosomes in the 26-chromosome species pairs with the 13-chromosome set from the American 13-chromosome species, whereas in the cross with the Asiatic species the large set pairs with the Asiatic 13-chromosome set. It appeared, however, that all the chromosomes were slightly larger in the triploid of American 26- ×Asiatic 13-chromosome. Crosses between 26- and 13-chromosome American species rarely had associations of more than two chromosomes, although associations of three were found. M I figures with 13 associations of two chromosomes and 13 univalents were common, but figures with less than 13 pairs were also present (Plate 1, fig. F). Several figures with 11 associations of two chromosomes and 17 univalents were found, and one or more fragments were present in some cells. Early A I usually showed two or three bridges, and late A I had about the same number. Figures were found with five bridges and evidence of others; but in some A I figures there was little evidence of bridges. Three $2(AD)\times 2D_2$ crosses used in this study—*G. hirsutum*, *G. barbadense*, and *G. hopi*×*G. harknessii*—had essentially the same chromosome behavior. A few observations of *G. hirsutum*×*G. thurberi*, $2D_1$, gave about the same range in the number of univalents.

Several crosses between 26- and 13-chromosome American species studied by SKOVSTED (1937) gave much the same chromosome behavior as that described above, except that he failed to mention the occurrence of chromosome bridges. In some M I figures he found more than 13 associations of two chromosomes. Associations of four chromosomes were rare. In rare figures there were only five to nine associations of two chromosomes. He found a chiasma frequency in bivalents slightly below that of the normal species. From SKOVSTED's data (table 1, group 12) and the present work, it appears that crosses in any combination of 26- and 13-chromosome American species have about the same meiotic chromosome behavior.

Hexaploids, $2[(AD)_1D_2]$, produced by doubling the chromosome number in sterile triploids of *G. hirsutum*×*G. harknessii*, have a higher fertility and, therefore, apparently a more normal chromosome behavior during meiosis than hexaploids, $2[(AD)_1A_1]$, of American 26- and Asiatic 13-chromosome species. HARLAND (1940) found two hexaploids of the former type to be "fully fertile." In most cells of the $2[(AD)_1D_2]$ type there are univalents, trivalents, associations of three or four chromosomes, or a combination of the three, and in some A I figures one or more bridges are present.

It may be said that the chromosome set in American 13-chromosome species is similar in structure to the small 13-chromosome set in American 26-chromosome species. There are, however, a number of structural differences, perhaps five or more.

American 13- ×Asiatic 13-Chromosome Species

Hybrids between 2A and 2D types, Asiatic 13- and American 13-chromosome species, were reported by SKOVSTED (1937) to average about eight associations of two chromosomes and nine to ten univalents at M I. An occasional association of three or four chromosomes was reported. Chiasma frequency in bivalents averaged less than 1.2 (table 1, group 4). His drawings show chromosome bridges. The only cross available for a study of this type in the present work was *G. arboreum* var. *neglectum*×*G. thurberi*. It was found that chromosome bridges were present in nearly every A I, the number ranging from 0 to 9 and averaging 4.7 (table 4). The difference between the present data and that of SKOVSTED may be explained on the basis that slightly different crosses were studied, or on the fact that the number of bridges varies with different environments, as shown in table 4.

TABLE 4

Chromosome bridges at A I of G. arboreum var. neglectum×G. thurberi.

MATERIAL COLLECTED	NUMBER BRIDGES PER CELL									
	0	1	2	3	4	5	6	7	8	9
Fall, 1937		2	1	6	8	13	12	8	10	2
Summer, 1938	2	6	2	10	3	3		1		
Fall, 1938		2	1	1	6	3	1	2		1
Total	2	10	4	17	17	19	13	11	10	3

Total number cells, 106
Average number bridges, 4.7

In an amphidiploid produced from the hybrid all the chromosomes pair usually as bivalents, but higher associations are frequently present. Some A I figures have no bridges, while others have one to three. If the bridges in the initial hybrid were a physiological effect of hybridization, there should be more attenuated chromosomes at A I in the tetraploid than there were in the initial hybrid.

From the number of univalents, the number of bridges in the F_1, and, the behavior of the chromosomes in the tetraploid produced by doubling the chromosome number in the F_1, it seems safe to conclude that structural differences exist between all the chromosomes of Asiatic 13- and those in American 13-chromosome species.

The tetraploid produced by doubling the chromosome number in a hybrid of Asiatic 13- ×American 13-chromosome species was crossed with American 26-chromosome species. At M I about one-third of the cells have one or two univalents, most of the remaining chromosomes are paired

as bivalents, but most cells have one or more multivalent associations. A cell with more than the average number of irregularities is shown in Plate 1, figure G. In some cells three multivalent associations were found, and sometimes bridges were seen in anaphase figures. MAUER (1938) reported meiosis to be regular in a similar hybrid. The number of multivalents and univalents found in the hybrid $A_2D_1(AD)_1$ supports the statements made earlier in this paper that three or more structural differences exist between American 26- and Asiatic 13-chromosome species of Gossypium and about five structural differences between American 26- and American 13-chromosome species.

American 26- ×Australian 13-Chromosome Species

A cross $2(AD)_1 \times 2C_1$, American 26- and Australian 13-chromosome types, *G. hirsutum*×*G. sturtii*, showed from one to nine, and averaged 5.0, chromosome bridges at A I (Plate 1, fig. H; table 5). Approximately 20 percent of the cells had associations of three chromosomes, but associations of four chromosomes were rare. According to SKOVSTED (table 1, group 10), the average number of bivalents in this cross is 7.6. One of his figures of a similar cross shows five bridges.

Hexaploids, $2[(AD)_1C_1]$, produced by doubling the chromosome number in triploid hybrids, sometimes have 39 pairs of chromosomes, although usually some are in associations of four, and two or more univalents may be present. Ordinarily one to three bridges are present at A I in this hexaploid (Plate 1, fig. I), but bridges are sometimes absent. The behavior of chromosomes in the triploid hybrid of these two species and in the hexaploid produced from the hybrid indicates that all the chromosomes of the two species are structurally differentiated.

TABLE 5

Frequency of bridges in a cross of G. hirsutum×*G. sturtii*, $2(AD)_1 \times 2C_1$.

Number bridges	1	2	3	4	5	6	7	8	9
Frequency	2	7	11	14	25	15	11	8	1

Total number cells, 94
Average number bridges, 5.0

American 26- ×African 13-Chromosome Species

A cross $2(AD)_2 \times 2B_1$, *G. barbadense*×*G. anomalum*, according to SKOVSTED (1937), had an average of 2.6 associations of two chromosomes and 33.8 univalents (table 1, group 8). The chiasma frequency was 1.05. SKOVSTED'S drawing of an A I of this cross has four bridges. WEBBER (1939) from a similar cross, *G. hirsutum*×*G. anomalum*, found the number of associations of two chromosomes to average 10.4.

In the present work the cross *G. barbadense* (Pima) × *G. anomalum* averaged 8.9 bivalents at first metaphase in 34 pollen mother cells. Most of the bivalents were attenuated in anaphase, which indicates that most of the chromosomes that pair have structural differences (Plate 1, fig. J).

A hexaploid $2[(AD)_1B_1]$ from *G. hirsutum* × *G. anomalum* was nearer normal in chromosome behavior than any of the other induced Gossypium polyploids that were studied (Plate 1, fig. K). No irregularities were observed in about half of the M I cells; in others, univalents were the most common irregularity, and more rarely multivalents were present. Sometimes one or more bridges were present at anaphase.

American 26- × Arabia-India 13-Chromosome Species

SKOVSTED reported the cross $2(AD)_2 \times 2E_1$, *G. barbadense* × *G. stocksii*, to average 37.9 univalents and 0.55 association of two chromosomes (table 1, group 13). The maximum number of associations of two chromosomes found was three. In the present work a hybrid of *G. hirsutum* × *G. stocksii*, $(AD)_1E_1$, in 71 PMC averaged 1.9 bridges at A I. A maximum of five bridges was found. The average number of bridges in this cross probably does not differ significantly from the average number found in haploids of *G. hirsutum*.

Asiatic 13- × Australian 13-Chromosome Species

In a cross $2A_2 \times 2C_1$, *G. arboreum* × *G. sturtii*, SKOVSTED reported M I figures to average 2.8 univalents, 9.8 associations of two, and the remainder as associations of three or four chromosomes. The number of univalents ranged from 0 to 14 (table 1, group 3). He determined the chiasma frequency as 1.3. His text figure 6 indicates that some of the bivalents form bridges at anaphase. WEBBER (table 1, group 3) from a similar cross $2A_1 \times 2C_1$, *G. herbaceum* × *G. sturtii*, reported the number of bivalents to average 2.2. These data show that considerable chromosome differentiation exists among the species.

Asiatic 13- × Arabia-India 13-Chromosome Species

SKOVSTED reported hybrids of $2E \times 2A$, *G. stocksii* × Asiatic species, to have from 4 to 26 univalents with an average of 14.9, and the number of associations of two chromosomes averaged 5.6 with a chiasma frequency of 1.1 (table 1, group 5). In the present work a cross of this type *G. stocksii* × *G. arboreum* at M I of 41 pollen mother cells gave a range of 12 to 26 univalents and averaged 3.8 associations of two chromosomes. Although some typical bridges were found and fragments were seen in a small number of cells, most of the associations of two chromosomes were not as attenuated as the bridges in other hybrids. Doubling the chromosome num-

ber in this hybrid gave a polyploid of almost normal fertility. Apparently there is little homology among the A and E sets of chromosomes.

Asiatic 13- ×African 13-Chromosome Species

In crosses between Asiatic 13- ×African 13-chromosome species, 2A×2B, SKOVSTED determined the average number of univalents as 2.2, of bivalents as 11.4, and found a few associations of three and four chromosomes. The range of univalents was 0 to 9 (table 1, group 2). WEBBER reported similar data. A hybrid *G. arboreum* var. *neglectum*×*G. anomalum* studied in this work gave essentially the same averages. There is much homology between A and B sets of chromosomes, but four or more structural differences exist.

Australian 13- ×American 13-Chromosome Species

WEBBER studied four 2D×2C crosses, American 13-×Australian 13-chromosome species, and SKOVSTED studied two of the crosses (table 1, group 9). WEBBER reported an average of about one association of two chromosomes, while SKOVSTED reported an average of about 8.5 associations of two chromosomes in his crosses. WEBBER and SKOVSTED show similar differences in their results from a study of *G. barbadense*×*G. sturtii* and for other hybrids. SKOVSTED's results on a similar cross, *G. hirsutum*×*G. sturtii*, agree with the author's data given earlier in this paper for that cross. It is believed the differences between WEBBER's data and that of other workers is the result of WEBBER's interpretation of chromosome bridges involving pairs of chromosomes as fragmenting univalents. The most logical way to interpret his figures, in the light of the present work, is to assume that the extra chromosome bodies in his drawings are fragments resulting from crossing over between relatively inverted segments of paired chromosomes. SKOVSTED's figures (table 1, group 9) show several bridges. From the data available there can be little doubt that every chromosome in *G. sturtii* differs in structure from every chromosome in American 13-chromosome species.

African 13- ×American 13-Chromosome Species

Crosses of 2D×2B, between three American 13-chromosome species and the African species, *G. anomalum*, were reported by SKOVSTED (table 1, group 7) to have an average number of univalents varying from 14 to 21, depending upon the American 13-chromosome species involved; the average number of associations of two chromosomes varied from 2.4 to 5.7. Associations of three chromosomes were rare. SKOVSTED's figures give evidence that bridges are formed at A I. WEBBER reported the number of associations of two chromosomes to average 0.2. AMIN (1940) reported a

hybrid of *G. davidsonii*×*G. anomalum* to be fertile after its chromosome number was doubled with colchicine. In a hybrid of *G. davidsonii*×*G. anomalum* the author found an average of 3.8 bivalents and a maximum of eight bridges. These data indicate that B and D sets are well differentiated.

Other Combinations

Crosses between some combinations have not been studied, for attempts to produce them either failed or the seedlings died before they reached the flowering stage (SKOVSTED 1935b). These combinations are 2E×2C, Arabia-India (*G. stocksii*)×Australian (*G. sturtii*); 2B×2E, African (*G. anomalum*)×Arabia-India; and 2C×2B, Australian×African.

RELATIONSHIPS BETWEEN THE TYPES OF GOSSYPIUM

In the section devoted to the cytological behavior of hybrids and polyploids, the available detailed cytological evidence was cited on the relationships of the species of Gossypium, but the details obscured the relationships within the genus as a whole.

Failure to secure crosses has prevented a cytological study of four combinations of crosses between the six types. For each absent combination it is possible, as pointed out by SKOVSTED (1937), to cite dubious evidence from triangular relationships of species. He stated, "Consequently it appears reasonable that two species B and C may have originated from a species A and developed in different directions, so that B and C ultimately lose more of their cytological homology with each other than with the parental species A." This is possible if the parent species changes at a slower rate than the derived species. The results may be invalid if two species arose from a common ancestor, and one of the species later branched to give a third. Since it is known that chromosome pairing is under genic control, it is dangerous to judge relationships from triangular associations alone.

In one triangle made up of Asiatic, Australian, and American 13-chromosome species the number of bivalents differs by a minimum of 3.3 in crosses between any combination of two of the three species, but in another triangle involving a different American 13-chromosome species the number of bivalents differs by as much as six (fig. 6). In the triangle, Asiatic, African, and American 13-chromosome species, the frequency of bivalents varies in the three crosses (fig. 6). It is obvious that triangular associations give little definite information about the relationship of species.

The relationships among the six types are summarized in figure 6. It is apparent that the types have different degrees of similarity. In some instances different combinations of crosses between members of two types

give different results. Crosses between members of a type show few or no chromosome structural differences. In the American 13-chromosome type the data are inadequate. The lack of evidence that the chromosomes of *G. davidsonii* and *G. klotzschianum* have several structural differences from the chromosomes of *G. thurberi*, *G. harknessii*, etc., is the reason the American 13-chromosome species are here considered as one type in place of two as suggested by HARLAND (1939).

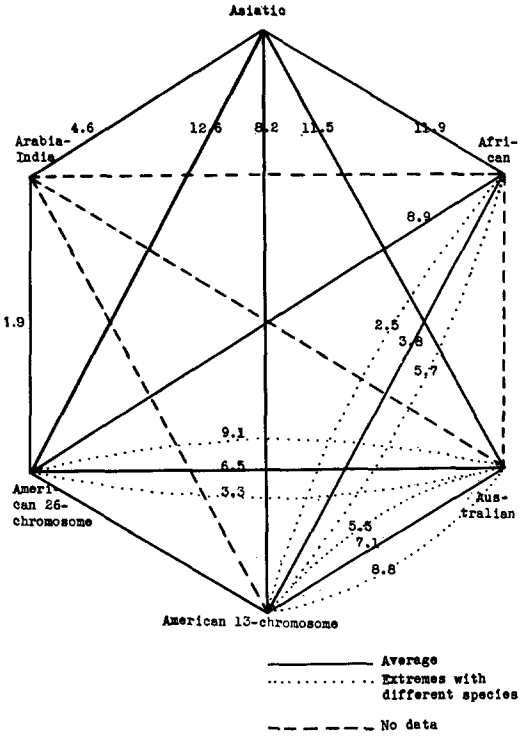

FIGURE 6.—Number of bivalents (number of chromosomes associated divided by two) in crosses between different types of Gossypium. Data from SKOVSTED (1933, 1935, 1937) except for American 26- × African, and American 26- × Arabia-India, which are from the present work.

As shown by an average of 2.4 univalents, 11.3 associations of two chromosomes, and a few associations of more than two chromosomes at M I, the Asiatic type, 2A, *G. arboreum* and *G. herbaceum*, and the African type, 2B, *G. anomalum*, have the closest relationship that exists among the six types. From SKOVSTED's data, the Asiatic type and the Australian type, 2C, *G. sturtii*, are slightly more distantly related, since there is an average of 2.8 univalents, 9.8 associations of two chromosomes, and a few multivalent associations. American 13-chromosome species, 2D, are next in closeness of relationship with Asiatic species, there being 9.6 univalents, 7.8 associations of two chromosomes, and rarely an association of three or

four chromosomes. An average of 4.7 bridges at A I shows that the paired chromosomes in the hybrid usually are structurally differentiated. Based on an average of 16.6 univalents and 4.6 associations of two chromosomes in hybrids between the Arabia-India type, 2E, *G. stocksii* × Asiatic species, it appears that *G. stocksii* is more distantly related to the Asiatic species than are the other types; on the other hand, if any consideration is given to the triangular relationships shown in figure 6, the Asiatic type is more closely related to *G. stocksii* than are any other types.

Although relationships of the African type, 2B, to Asiatic species, 2A, are the closest of any of the types, the differences are particularly apparent in crosses involving the two species with a third. For example, *G. anomalum*, 2B, averaged 18.5 univalents and 3.8 associations of two chromosomes in crosses with American 13-chromosome species, 2D, while the comparable number of bivalents in 2A × 2D crosses was 8.2 (fig. 6). Further, African × American 26-chromosome crosses averaged 3.6 bivalents less than American 26-chromosome × Asiatic.

The Australian type, 2C, *G. sturtii*, averaged 8.2 and 5.3 associations of two chromosomes at M I in crosses with different American 13-chromosome species, 2D. *G. sturtii* is doubtlessly more closely related to Asiatic species than to American 13-chromosome species. The relationship between Australian and Arabia-India species, 2E, is apparently distant, and it may approach the differences existing between *G. stocksii* and Asiatic species plus the differences between *G. sturtii* and Asiatic species (fig. 6).

There are indications that the degree of relationship of American 13-chromosome species, 2D, with Australian, 2C, Asiatic, 2A, and African species, 2B, decreases in the order given. There is no direct evidence available on the relationship of American 13-chromosome species with the Arabia-India species, 2E, *G. stocksii;* however, it is possible that these two are the most distantly related of the six types. The evidence for this is an average of only 1.9 associations of two chromosomes in a cross of *G. stocksii* with an American 26-chromosome species, 2(AD).

SKOVSTED (1937) divided 13-chromosome Gossypium species into an American and an Old World group. WEBBER (1939) separated the Old World group into Australian and Asiatic. The data shown in figure 6 and the relationships pointed out above show it to be erroneous to include all Old World species in one or even in two types. There is evidence of less homology between chromosomes of certain of SKOVSTED'S (1937) and WEBBER'S (1939) Old World types than there is between some Old World types and New World species. For example, the average number of associations of two chromosomes in crosses between the Arabia-India, 2E, species, *G. stocksii*, and Asiatic, 2A, species is 4.6, while the number in a cross between American 13-chromosome species, 2D, and Asiatic species is 8.2.

The relationship and origin of American 26-chromosome species remains to be considered. As shown in a previous section, two 13-chromosome sets in hybrids between American 2(AD) 26- and Asiatic, 2A, 13-chromosome species tend to associate as bivalents or higher multiples. The larger 13-chromosome set in American 26-chromosome species pairs with the set of similar size from Asiatic species. There is, however, a minimum of three structural differences in chromosomes between the two sets that pair. In hybrids of 26-chromosome species with an autotetraploid Asiatic type, the chromosomes in three 13-chromosome sets tend to associate as trivalents.

In crosses between American 26- and American 13-chromosome species, two 13-chromosome sets tend to associate as bivalents; but in this case it is the smaller 13-chromosome set that associates with a set of similar size from an American 13-chromosome species. There is a minimum of four structural differences between the sets of chromosomes that pair.

These facts, summarizing chromosome behavior in hybrids between American 26-chromosome and Asiatic and in hybrids between American 26-chromosome and American 13-chromosome types, except for the structural differences between the chromosomes that pair, were noted by SKOVSTED (1934). He advanced a hypothesis that American 26-chromosome species are allotetraploids, which arose from a hybrid between American 13- and Asiatic 13-chromosome species. SKOVSTED (1937) reported an average of 7.8 bivalents in a hybrid between Asiatic 13- and American 13-chromosome species. GATES (1938) cast some doubt on SKOVSTED's hypothesis and suggested that 26-chromosome cottons could be either autotetraploids or allotetraploids involving two American 13-chromosome species. WEBBER (1939) discussed the published evidence of the origin of American 26-chromosome cottons.

Obviously, the most critical evidence in this matter is the pairing of the two sets of chromosomes in American 26-chromosome species in crosses with tetraploids synthesized from an American 13- and an Asiatic 13-chromosome species (BEASLEY 1940b). Since usually only one or two, and frequently no, univalents are present at M I in hybrids of this type and the hybrids are partly fertile, there is no doubt that one set of chromosomes in the American tetraploid 26-chromosome species is similar in structure to the set in American 13-chromosome species and that the other set is similar to the set in cultivated Asiatic species. As SKOVSTED (1934) doubtlessly appreciated, and as pointed out by MAUER (1938), there is no evidence that any existing 13-chromosome species is exactly like those involved in American tetraploid species.

MAUER, from geographic distribution and genetic differences among the 26-chromosome species of Gossypium, suggested independent origins for

the species and that different 13-chromosome species were the parents of the allotetraploids. Geographical distribution does not support the idea of separate origins, for extremes of the tetraploid type are native in the New World. The behavior of the meiotic chromosomes of the tetraploid synthesized from species similar to the ones that gave rise to the American 26-chromosome species strongly supports the idea that all the natural tetraploid Gossypium species came from one original tetraploid plant. The evidence is that the synthesized tetraploid has a rather high frequency of chromosome irregularities. If separate lines were established from the initial progeny of the tetraploid, they would probably differ in chromosome structure in a manner similar to the different lines of Triticale examined by MÜNTZING (1939). The fact that meiotic chromosome behavior is regular in hybrids between the natural tetraploids is excellent evidence that the natural 26-chromosome species not only had a common origin but also that a single line from the original tetraploid was evolved with regular chromosome behavior and it later branched to give the existing natural tetraploid species.

The relationships of Gossypium species give an indication of the center of origin or distribution of the genus. Asia is suggested, for Asiatic species are apparently intermediate in the relationships among other types. To judge from the available evidence, the Arabia-India type is more closely related to Asiatic species than to other types. It is, however, more distantly related to Asiatic species than Asiatic species are to other types. The Australian, African, and American 13-chromosome types are more closely related to Asiatic species than to each other, with the possible exception that American 13-chromosome cottons are about equally distantly related to Asiatic and Australian types.

CHANGES IN CHROMOSOME STRUCTURE AND SPECIATION

Ample evidence has been given to show that there are many differences in chromosome structure among the types of Gossypium. For example, pollen mother cells were found with nine bridges (involving 18 chromosomes) in the cross *G. arboreum* var. *neglectum*×*G. thurberi*. It is probable that the other eight chromosomes failed to pair because the structural differences between them were too numerous. The maximum number of bridges found in a cross between the Australian and the American 26-chromosome type was also nine.

It is the author's opinion that in any diploid hybrid that shows half or less than half of the chromosomes in associations of two, the bivalents will usually give bridges at anaphase, for the reduction in the number of bivalents is believed to result usually from structural differences between

chromosomes (DOBZHANSKY 1937). It follows that in hybrids with a sufficient number of structural differences to cause the failure of half the chromosomes to pair, it is probable that one or more structural differences exists between paired chromosomes. Gene combinations, asynaptic genes, are known to prevent or greatly reduce chromosome pairing, but that type of chromosome behavior is not considered to hold here, because the pairing of the chromosomes in polyploids from the hybrids is almost normal. Doubling the chromosome number in asynaptic plants is not followed by normal chromosome pairing (unpublished data).

The extent of structural differences in the chromosomes of different species of Gossypium may be divided into four classes. The first class includes types lacking any apparent differences in chromosome structure; for example, *G. hirsutum*, $2(AD)_1 \times G.$ *barbadense*, $2(AD)_2$. The second class includes types that approach the normal number of associations, but a few bridges, univalents, or multivalents, or combinations of the three, are present at A I. An example of this type is *G. arboreum* var. *neglectum* $2A \times G.$ *anomalum*, 2B. In the next type there is a reduced and variable number of associations of two chromosomes, and nearly every chromosome association gives a bridge at A I. The cross *G. hirsutum*, $2(AD), \times G.$ *sturtii*, 2C (table 1, group 10, and table 5), is an example. The fourth class consists of species crosses in which the structural differences among chromosomes are so numerous that almost no chromosome association is present, as illustrated by *G. hirsutum*, $2(AD), \times G.$ *stocksii*, 2E.

The present problem is to evaluate the importance of chromosome structural changes in the speciation of Gossypium and to attempt to account for the existence of the changes.

It may be that chromosome structural changes are of incidental importance compared to gene mutation and that similar species differences would exist today if the chromosomes had undergone no rearrangement, provided the species had remained isolated by other factors and polyploid species were excluded. Some evidence that speciation in Gossypium is independent of chromosome structural changes is seen from the species grouped within the general types. These species show little chromosome differentiation, yet they differ so greatly in leaf size, shape, flowers, physiology, etc., that there is no doubt of their being distinct species. The American 13-chromosome type contains species that were originally classified into three genera: *G. thurberi* Tod. = *Thurberi thespesiodes* A. Gray; *G. armourianum* Kearney = *G. armourianum* Kearney; *G. aridum* (Rose and Standley) Skovsted = *Erioxylum aridum* Rose and Standley. The American 26-chromosome type has species ranging from small herbs to perennial shrubs or trees, and they show other outstanding differences, yet in crosses between

species, they are all inter-fertile and exhibit almost normal chromosome behavior. In these cases isolation was probably geographic, and gene changes were of major importance in the differentiation of species.

There appear to be differences in chromosome structure between each of the six types of Gossypium, but, since the American 26- and the American 13-chromosome types include distinct species with few or no structural differences among their chromosomes, it appears that most of the structural differences that exist among the six types became established after the species were separated. The apparent fact that initial speciation in Gossypium is by gene changes does not mean that changes in chromosome structure were unimportant in the speciation of the genus. The appearance of an allotetraploid type is probably the greatest single step in speciation of Gossypium, and this could hardly have occurred without chromosome structural differentiation among species.

Since chromosome structural changes in Gossypium are seemingly of little importance in initial speciation, it may be assumed that they are unimportant in the evolution of a single species. It is probable that gene changes arise and become established at a much higher rate than chromosome changes, which gives them greater initial importance. It is likely, however, that chromosome changes occur that are also important steps in the evolution of a species, for, as pointed out by DARLINGTON (1937, 1940), they could give types of variation (usually duplication of genes and perhaps position effect) that could hardly arise by other means.

The work of DOBZHANSKY and STURTEVANT (DOBZHANSKY 1937) shows that changes in chromosome structure are frequent in Drosophila and that they are important in the evolution of species. STEBBINS (1938) found changes in chromosome structure to be important in the isolation of species, but if species were isolated by other means, as great or greater species differences could exist without changes in chromosome structure.

At present it is a question how the chromosome structural changes in Gossypium became established. An initial structural change should be a disadvantage, since it would be heterozygous, and many of the gametes would be inviable. Since there is usually more self- than cross-pollination in Gossypium, individuals homozygous for the chromosome change could be produced, but the type would be in a minority; and unless changes in chromosome structure possessed survival value, the type ordinarily would be lost, or it would be carried along in the population like a gene with no survival value. If the population were small and large fluctuations were frequent, at times by chance structurally changed types would replace original types. The survival of types by chance, rather than by survival of the fittest, is discussed by DOBZHANSKY (1937).

Structural changes have occurred in the chromosomes of the six types of Gossypium mentioned above, and they have survived and supplanted earlier chromosome types. The rate at which this chromosome differentiation can become established is slow. This in no way means that changes in chromosome structure are rare, for the frequency with which they are found in nature shows that they are relatively common (SANSOME and PHILP 1939). The rapidity with which structural changes are incorporated into species is an entirely different matter. If changes in chromosome structure possess survival value, the selection pressure for their survival, after they become homozygous in a species that is mostly self-pollinated, would be the same as that for a gene of equal survival value.

These structural chromosome changes have a significance in regard to the supposition that 13-chromosome cottons are primitive polyploids. This idea was advanced by DAVIE (1935) and by WEBBER (1935) and was supported by SKOVSTED (1937). The idea could be correct, but basing the argument on the secondary pairing found in some 13-chromosome cottons is objectionable. Obviously, since so many structural changes have occurred in Gossypium, it is almost certain that there are duplications within a basic chromosome set. The autosyndesis reported by SKOVSTED (1935) is cytological evidence that duplications are present. These duplications could be the cause of much secondary pairing. DARLINGTON (1937) gives a general discussion of such possibilities.

Considering the evidence available, it appears that the first step in the speciation of Gossypium is the geographical or physiological isolation from the general population. Gene mutation and different gene combinations arise rapidly, and some of them become established at a comparatively rapid rate. Detectable structural changes in the chromosomes arise occasionally, and some of them become established, but at a much slower rate.

SUMMARY

The meiotic chromosome behavior is regular in 13-chromosome species of Gossypium, but there are a few irregularities in 26-chromosome species. In meiosis of autopolyploids about two-thirds of the chromosomes form quadrivalents. In a haploid of a 26-chromosome species a maximum of five pairs of chromosomes was found.

In hybrids between species the amount of chromosome pairing varies from complete to almost none. In hybrids with a reduced amount of pairing there was evidence that structural differences existed among some of the chromosomes, and in some species hybrids apparently all the chromosomes were structurally dissimilar. Most of the chromosomes formed bivalents in polyploids that were produced from hybrids with a reduced

amount of chromosome pairing; usually, however, cells in first metaphase and anaphase had one or more anomalies.

The tetraploid species of Gossypium have one set of chromosomes similar to the set in Asiatic 13-chromosome species and the other set similar to the set in American 13-chromosome species.

The species of Gossypium are separated into six general types chiefly on the basis of chromosome pairing, structure (arrangement of the genes), and chromosome number. The degree of relationship of the types is discussed.

Structural changes in the chromosomes probably had little importance in the initial speciation of Gossypium.

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