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# A Reexamination of the Diploidlike Meiotic Behavior of Polyploid Cotton

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Summary. Chromosome associations at pachytene, diakinesis, and metaphase I were analyzed in haploids of the allotetraploid *G.hirsutum*, in the $F_1$ hybrid between *G.arboreum* ($A_2$) and *G.raimondii* ($D_5$), and in the doubled hybrid $2(A_2D_5)$ in an effort to define more clearly the mechanism responsible for the diploidlike behavior of the natural allotetraploids. The mean number of bivalents per cell at pachytene, diakinesis, and MI were respectively 10.00, 7.40, and 0.80 for the haploids and 11.00, 9.50, and 5.82 for the $A_2D_5$ hybrid. The two pachytene means were not significantly different, but the two diakinesis means and the two MI means were significantly different.

At early pachytene members of paired chromosomes were not equal in length, but at late pachytene both members were equal in length in most bivalents. It was particularly evident in the unpaired regions that one partner was much thicker or more deeply stained than its counterpart. Since A chromosomes are twice the size, have twice the amount of DNA, and contain greater amounts of repetitive DNA than D chromosomes, it was concluded that the bivalents consisted of A and D homoeologs. In order for the paired homoeologs to attain equality in length at late pachytene, it is presumed that the A chromosomes either started contracting before the D chromosomes or contracted at a faster rate. During the stages following late pachytene, the D chromosomes contracted at a faster rate than the A chromosomes resulting finally in a two-fold difference in the size of the A and D chromosomes at MI. It is assumed that this differential rate of contraction would limit intimate pairing attraction and chiasma formation between homoeologous chromosomes.

We concluded that a gene control system analogous to that wheat and oats does not determine the diploidlike meiotic behavior of the natural allotetraploids of *Gossypium*. This is based on the high frequency of homoelogous pairs at pachytene in the haploids, on the strict homologous pairing in the synthetic $2(A_2D_5)$ hybrid, and on the roles repetitive DNA is assumed to have in regulating synapsis and chiasma formation and in structurally differentiating the A and D homoeologs.

It was proposed that differences in genome chromosome size may determine whether a gene or non-gene control mechanism is required for regulating homologous pairing in allotetraploids.

## Introduction

The diploid species (2n = 2x = 26) of *Gossypium* have been divided into six genome groups, two of which are designated A and D. The allotetraploid (2n = 4x = 52) species are made up of A and D genomes that are highly homoeologous to the A and D genomes of the diploids.

The metaphase I chromosomes of the A and D genomes of both the diploids and the allotetraploids are different in size, the A chromosomes being about twice the size of the D chromosomes. Brown (1954, 1958, 1961, 1962), however, reported that pachytene AD bivalents in haploids of *G.hirsutum* and in species hybrids were of the same length despite their differential size in the ensuing MI. At that time, these two observations were not considered contradictory since it was assumed that polynemy could account for the difference in chromosome size at MI.

Kimber (1961) proposed a genetical mechanism analogous to that in wheat to account for the diploidlike pairing behavior in the amphidiploid cottons. Endrizzi (1962) and Gerstel (1966) suggested alternatives to Kimber's genetical mechanism. The former author based his proposal on the difference of chromosome size of the A and D genomes and on Brown's pachytene pairing observations. He proposed that the differences in linear movement or contraction between the A and D chromosomes of the allotetraploids was the major force regulating homologous pairing. Although the evidence is not entirely one-sided, it does indicate that a controlling mechanism like that in wheat does not occur in the cotton amphidiploids (Riley and Law, 1965). Nevertheless, the allotetraploid cottons are often cited as a possible case for gene controlled mechanism for regulating chromosome pairing. The present report discusses additional information obtained in *Gossypium* which reinforces the premise for the non-gene model of control of bivalent pairing in *Gossypium*.

Following those earlier cytological studies in *Gossypium*, it has been shown that the size difference of the MI chromosomes of the A and D genomes

Table 1. Chromosome associations at pachytene, diakinesis and metaphase I in *Gossypium hirsutum* haploids, $(AD)_1$, and *G. arboreum* ($A_2$) × *G. raimondii* ($D_5$) hybrids, $F_1A_2D_5$ and $2(A_2D_5)$

| | $(AD)_1$ Hyploid | | $F_1 A_2D_5$ | | $2(A_2D_5)$ | |
|---|---|---|---|---|---|---|
| Meiotic Stage | Number of Cells | Mean No. of Bivalents per Cell and Range | Number of Cells | Mean No. of Bivalents per Cell and Range | Number of Cells | Mean No. of Bivalents per Cell |
| Rachytene | 13 | 10.00(4-12) | 5 | 11.00(9-12) | 12 | 26* |
| Diakinesis | 103 | 7.40(4-11) | 102 | 9.50(6-12) | 60 | 26 |
| Metaphase I | 2070 | 0.80(0-7) | 320 | 5.82(1-11) | 194 | 25.78 |

* Estimated by comparing its pachytene pairing with that of the natural allotetraploid *G. hirsutum* and that of the $(AD)_1$ hyploids and $F_1 A_2D_5$ hybrid.

correspond to their difference in DNA content (Katterman and Ergle 1970; Edwards et al., 1974). Furthermore, recent studies of renaturation rate curves of denatured DNA show that the amount of repetitive DNA is directly related to chromosome size in these two genomes (Wilson 1974). In view of the overwhelming evidence for the uninemic structure for the eukaryotic chromosome, these data suggest that the A and D homoeologs should differ in length at pachytene. Brown's reports would appear, therefore, to be at variance with these more recent findings.

Lacking in the earlier cytological studies of *Gossypium* haploids and of $F_1$AD hybrids was a detailed examination of the early and late stages of pachytene. Such observation would serve a two-fold purpose: To recheck Brown's observations in an effort to reconcile the discrepancy between DNA content of A and D chromosomes and their pachytene length in AD bivalents and to use this information along with recent findings in an attempt to define more clearly the nature of the mechanism regulating homologous pairing in the natural allopolyploids of *Gossypium*.

## Materials and Methods

Plant materials used were five haploids [$(AD)_1$ genome, n = 2x = 26], each originating from a different genetic background of the allotetraploid *G. hirsutum*; $F_1$ hybrid (2n = 2x = 26) of *G. arboreum* ($A_2$ genome) × *G. raimondii* ($D_5$ genome), and its raw synthetic allotetraploid $F_1$ hybrid [$2(A_2D_5)$, 2n = 4x = 52]. The $F_1A_2D_5$ and $2(A_2D_5)$ hybrids used in the present study originated from the same hybrid plants studied by Endrizzi and Phillips (1960). The A genome of *G. arboreum* and D genome of *G. raimondii* are very closely related to the two taxons that contributed the A and D genomes of the natural allotetraploids (Phillips 1963). All plants were maintained in a glasshouse from which flower buds were collected and analyzed over a period of two years. The buds were killed and fixed in a 7:3 mixture of 95% ethyl alcohol and glacial acetic acid. Pachytene, diakinesis, and metaphase I stages were studied in iron-propinio-carmine smears of microsporocytes.

Diakinesis and MI stages are not too difficult to obtain in *Gossypium*. The pachytene stages, however, are less readily obtainable, and when found, are generally difficult to analyze; nevertheless, over 500 cells, mainly of the $(AD)_1$ haploids and the $A_2D_5$ hybrid, were studied. In a vast majority of these cells, analysis of the total complement was not possible due to the large number of chromosomes present and the frequent fusion of heterochromatic regions that prevented the identity of every single chromosome with certainty. Consequently, a complete, unambiguous pachytene analysis was obtained only in 13 cells of the haploids and in 5 cells of the $A_2D_5$ hybrid. We are confident that the results obtained with these cells represent the normal pachytene pairing behavior in the two plant forms because the results obtained from the partially analyzed cells showed ranges of pairing approaching that found in the completely analyzed cells.

## Results and Discussion

### Description of the Associated Chromosomes

The mean number of bivalents per cell and their ranges at pachytene, diakinesis, and MI in the three plant forms are given in Table 1. In the $(AD)_1$ haploids and the $A_2D_5$ hybrid the paired bodies were asymmetrical at meiotic prophase and MI. At early pachytene the paired chromosomes in both plant forms were either completely synapsed or incompletely synapsed, and the two chromosomes were not of the same length (Fig. 1a-f). Pachytene pairing in $A_2D_5$ was more intimate and extensive than in the $(AD)_1$ haploids. In both plant types the unpaired regions were usually one or two per association, and they could be short or long, terminal or intercalary (Fig. 1a-f). The intercalary unpaired segments were more frequent than the heteromorphic terminal ends. When the unpaired region was intercalary, two situations were encoun-

tered. The first involved short unpaired regions in which the partners appeared near equal in length, but one partner was much thicker or more deeply stained than its counterpart (Fig. 1a, b). The second situation was noted in the long unpaired regions in which the partners were markedly of unequal length in the differential segment (Fig. 1a, b, c). Here frequently the longer chromosome was thicker or more deeply stained and frequently contained loops and/or "fold-back" pairing (Fig. 1a, b). Heteromorphic terminals, unpaired (Fig. 1c, d, e, f) or paired (Fig. 1f), were not uncommon. Occasionally the longer member was thicker or more deeply stained than its shorter counterpart (Fig. 1e). In all the above situations, the synapsed regions preceding the unpaired region were often highly heterochromatic. It is assumed that the difference in length and thickness of the unpaired regions in bivalents in conjunction with the high frequency of bivalents per cell are indicative that they are heterogenetic associations, presumably between homoeologs, of A and D chromosomes.

At late pachytene most bivalents showed complete or normal pairing and both members of a pair were virtually of the same length (Fig. 1c, d, f). Some bivalents exhibited either bumps, loops, and overlaps. This equality in length of the two paired chromosomes at late pachytene is in marked contrast to their nonequality in length observed at early pachytene, suggesting differential behavior in contraction of members of paired homoeologs.

The results reported here are not in complete agreement with Brown's observations in which she reported that pachytene chromosomes can pair intimately and are of equal length irrespective of their size difference in the following metaphase. The two observations may in fact agree if it is assumed that her observations were made at late pachytene.

Pachytene pairing in the $2(A_2D_5)$ hybrid was complete and normal like that in the natural allotetraploid *G. hirsutum*.

Unpaired terminal segments and loops observed in the present materials are typical of those observed in hybrid combining two genomes with chromosomes of different lengths such as *Lolium perenne* × A. *temulentum* (Rees et al., 1966; Rees and Jones 1967), *Allium cepa* × A. *fistulosum* (Jones and Rees, 1968), and *Oryza sativa* × *O. australiensis* (Li et al., 1963). In the latter hybrid, the 12 chromosomes of *O. australiensis* are distinctly larger than the 12 chromosomes of *O. sativa*, and in the meiotic stages the two chromosome complements exhibited differential condensation and staining properties. As in the plant forms studied here, the large chromosomes of *O. australiensis* were stained more darkly and thicker in width even during the stage when the paired members were equal in

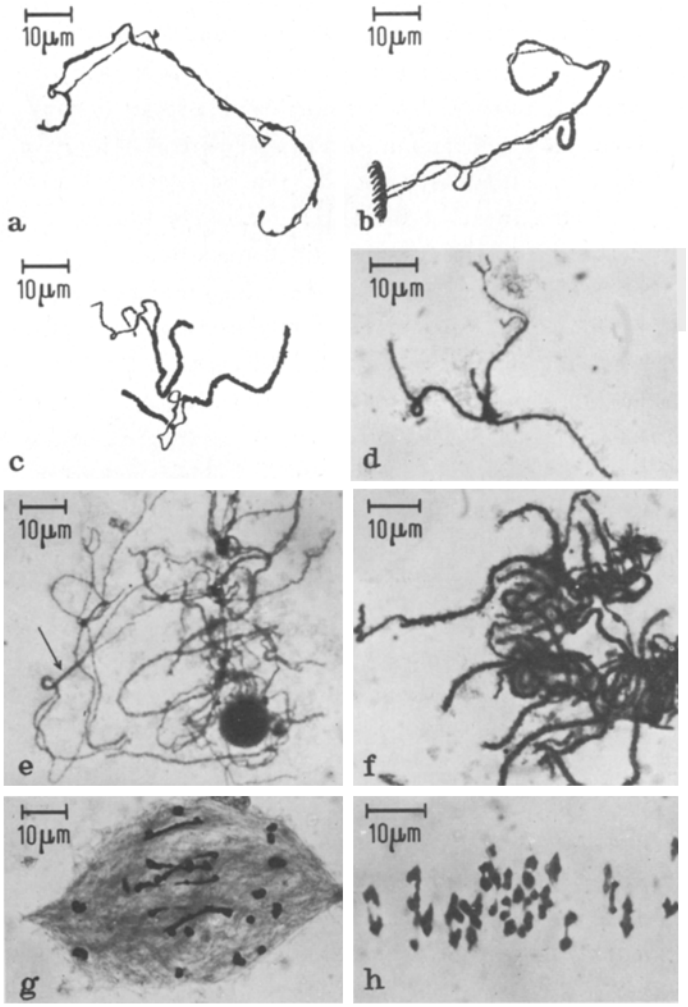


Fig. 1a-h. Pachytene and Metaphase I meiotic configurations of *Gossypium hirsutum* $(AD)_1$ haploids and *G. arboreum* $(A_2)$ × *G. raimondii* $(D_5)$ hybrid. (a) Early pachytene bivalent of $F_1\ A_2D_5$. (b-f) Early and late pachytene bivalents of *G. hirsutum* haploids. Intercalary and terminal unpaired heteromorphic regions are shown in Fig. 1a-f. Note nonequality in length and difference in thickness of paired members in the pachytene configurations. Also note "foldback" pairing in Fig. 1a, b, d. The arrow in Fig. 1e points to a single heteromorphic bivalent. Fig. 1a-c are camera lucida ink drawings, the others are photomicrographs. (g) shows MI pairing of 6II + 14I in the $F_1\ A_2D_5$ hybrid. Heterogenetic pairing of A and D homoeologs can be seen in the bivalents. Note that the large A chromosomes are twice the size of the small D chromosome. (h) MI of the $2(A_2D_5)$ hybrid showing 26II.

length. The difference in size of the chromosomes of the two genomes and their differential behavior in condensation (size and differential condensation are probably interdependent) may be of distinct importance as a mechanism to engender homogenetic pairing in an alloploid form.

At diakinesis in the $(AD)_1$ haploid and in the $A_2D_5$ hybrid, the chromosome size difference between A and D univalents and between paired members of AD bivalents was quite evident. The bivalents were asymmetrical, indicating heterogenetic pairing. The A chromosomes were more deeply stained than the D chromosomes and they were about two times larger. Some of the D univalents were very faintly stained, making them less conspicuous. Most bivalents were of the open type, however, closed bivalents were not uncommon. At diakinesis in the $2(A_2D_5)$ hybrid, all bivalents were ring-shaped (closed) and symmetrical, indicating strict homogenetic pairing.

Data on chromosome pairing at MI for AD haploids and for $A_2D_5$ hybrids have been reported by several investigators, therefore, we will only present our MI observations that are pertinent to the discussion. At MI, the A chromosomes are about twice the size of the D chromosomes (Fig. 1g). The bivalents in both plant forms exhibit heterogenetic pairing as evident by their asymmetry, and the majority were open or rod-shaped. Closed bivalents were far less frequent in the $(AD)_1$ haploids than in the $A_2D_5$ hybrid.

The $2(A_2D_5)$ hybrid at MI showed strict pairing of homologs in which 98% of the bivalents were closed. Eighty-five percent of the cells had the maximum of 26 bivalents (Fig. 1h) and the remaining 15% had 25 bivalents plus two univalents.

It was pointed out above that members of paired chromosomes were different in length at early pachytene, but that both paired members had attained similarity in length at late pachytene. Since the chromosomes of the A genome contain nearly twice as much DNA as chromosomes of the D genome, it is assumed that the longer chromosomes of the early pachytene pairs are A chromosomes. Thus, to attain equality in length at late pachytene the A chromosomes either started contracting before the D chromosomes or contracted at a faster rate. During the stages following pachytene the D chromosomes contracted at a faster rate than the A chromosomes, resulting finally in the two-fold difference in size in the A and D chromosomes at MI. We can assume that this differential rate of contraction of the chromosomes of the two genomes would have a significant influence on the degree of intimate pairing and on the formation of chiasmata between homoeologs. Differential rates of contraction between chromosomes and chromosome arms have been reported in other organisms (Ho and Kasha, 1974).

## Comparison of Chromosome Associations at the Meiotic Stages

The means and ranges of bivalents per cell at pachytene for the $(AD)_1$ haploids and for the $A_2D_5$ hybrid were 10(4-12) and 11(9-12), respectively (Table 1). The two means are not significantly different. In both plant forms, a few cells were observed in which chromosome associations indicated the maximum of 13 synapsed bodies were present. These results show that both plant forms have the same frequency of high heterogenetic pairing at pachytene. This suggests that the affinity existing between A and D homoeologs of the haploids and between $A_2$ and $D_5$ homoeologs of the hybrid are very similar and, presumably, comparable to that which existed between the two ancestral A and D genomes that combined to form the allotetraploid species. As meiosis progressed there was a decrease in the average number of bivalents per cell at diakinesis and at MI in both forms. The decrease was greater to a pronounced degree in the $(AD)_1$ haploids where the high mean of 10 pairs at pachytene was reduced to 7.40 at diakinesis and finally to 0.80 at MI. These values in the $F_1A_2D_5$ hybrid were respectively 11.00, 9.50, and 5.82. The frequency of bivalents at both diakinesis and MI of the $(AD)_1$ haploids were significantly different from those of the $A_2D_5$ hybrid. These values show that percent pairing in the haploids decreased from 77% at pachytene to a low between 1% and 8% at MI, while in $A_2D_5$, the decrease was from 85% at pachytene to 45% at MI. The high frequency of MI pairing in $A_2D_5$ is analogous to the high pairing recorded in diploid hybrids obtained from crosses of any two of the three diploids considered to be ancestors of hexaploid wheat (Riley and Chapman, 1958; Kimber and Riley, 1963). In hexaploid wheat the failure of pairing between its three genomes is now controlled genetically.

## Mechanism of Control of Meiotic Behavior in the Allotetraploids

Two important facts suggest to us that the near total elimination of bivalent formation in the haploids and the regular homologous pairing in the natural allotetraploids do not necessarily indicate that a gene control mechanism analogous to that in wheat exists in the allotetraploids.

The first is the high frequency of pachytene pairing in the $(AD)_1$ haploids, a frequency of occurrence equal to that of the $A_2D_5$ hybrid. In the latter, the two genomes undoubtedly arose from a common ancestral genome even though both species now occur on separate continents. Because of their common origin, a high frequency of bivalents can be expected in their $F_1$ since their chromosomes are homoeologous and a gene(s) preventing homoeologous pairing would not be expected in their genomes. Kimber (1962) studied prophase pairing in euhaploids and nulli-5B haploids of wheat and noted very little synapsis of homoeologs in the former. The nulli-5B haploids however, exhibited much more prophase pairing and complex configurations were frequently seen. He concluded that the action of the gene on chromosome 5B prohibits synapsis between homoeologous chromosomes. If the strict homologous pairing that occurs in the natural allotetraploids of *Gossypium* is genetically controlled as in wheat or oats, one would expect to find, as has been observed in wheat haploids, little or no pairing at pachytene in their haploids. The high frequency of pachytene pairing in the cotton haploids and the absence of pachytene pairing in wheat haploids argues against any kind of single-step genetic changes in the *Gossypium* allotetraploids as a mechanism restricting pairing to homologous chromosomes.

The second important fact is the strict homologous pairing occurring in the $2(A_2D_5)$ allotetraploid hybrid. The $A_2D_5$ hybrid showed an average of 5.8 AD bivalents at MI, but upon doubling the two genomes the resulting allotetraploid exhibited strict homologous pairing (Table 1), establishing that a genetically controlled mechanism would not be necessary for diploidization of chromosome pairing in the allotetraploids of *Gossypium*. This point has been emphasized for the bivalent-forming synthetic tetraploid, $2(A_2D_1)$ (Endrizzi 1962; Gerstel 1966). Other synthetic intergenomic tetraploids in *Gossypium* also show the shift to homogenetic association. Although only a few such tetraploids have been studied, the shift is most apparent in the hybrids involving genomes of different sizes; the intragenomic tetraploids, on the other hand, exhibit higher multivalent frequencies (Brown 1951; Sarvella 1958; Anonymous 1968).

One might argue, however, that the regular homologous pairing in these synthetic tetraploids, particularly in the 2 AD hybrids, could as well be due to structural changes that have accumulated in the contemporary diploids (Sears, in Gerstel 1966); the presumed structural changes would not have been present in their ancestral diploids from which the natural allotetraploids were derived. Data which argue against this interpretation have been reported by Sarvella, 1958; Gerstel and Phillips, 1958; Phillips 1962, 1963, 1964; Gerstel 1966.

The results of their studies show an autotetraploid-like behavior for multivalent frequency and for testcross segregation ratio of genetic markers in the synthetic hexaploids of *G. hirsutum* × Asiatic species (AD × A, tetrasomic for the A genome). This indicates that the A genomes of the diploid species and the allotetraploid species are structurally very similar. Of the nine diploid D species, the genome of *raimondii* ($D_5$) is most closely related to the D genome of the allotetraploid. In AD × $D_5$ hexaploids (tetrasomic for the D genome), multivalent frequency was lower than expected from random chromosome association (6.16 vs expected of 7.8 to 9.1) and testcross segregation ratios were higher than expected (average of 9.3:1 vs expected of 7:1, see Endrizzi 1974, for basis of the expected of 7:1 rather than the 5:1 ratio). Overall, these data suggest a high degree of structural similarity between the chromosomes of the A genomes and between the chromosomes of the two D genomes. They do not suggest a level of structural divergence sufficient to account for the homologous pairing that occurs in the synthetic tetraploids, unless one assumes the unlikely event of concomitant, parallel structural divergence of the two genomes of the allotetraploids and the contemporary diploids. It should be understood, that the structural changes discussed above are of the types, cryptic in nature, discussed by Stebbins (1950). Below we present data for the existence of different kinds of chromosome structural differentiation, i.e. differences in amounts of repetitive DNA, which we believe plays a significant

role in the regular bivalent formation of the allotetraploid.

The approximate 1:2 size relationship of the chromosomes of the D and A genomes (Fig. 1g) is apparent in both the $(AD)_1$ haploids and the $A_2D_5$ hybrid. Additional information of their chromosomes size relationship has been reported by Edwards et al. (1974). They also reported that the A genome contains nearly twice the amount of DNA as that of the D genome, and concluded that this increase in DNA content can be attributed to amplification of the repetitive sequences throughout the genome. Their conclusion on the interspersive pattern was confirmed very recently by Walbot (1975), who reported that the allotetraploid *G.hirsutum* consists primarily of interspersed repetitive and nonrepetitive sequence elements. Six homoeologous genetic maps have been established in the allotetraploid *G.hirsutum* (Edwards et al., 1974). Except for an inverted region, the maps reveal that gene order and map distances of homoelogs are virtually the same despite their two-fold difference in DNA content. This led Edwards et al. (1974) to conclude that the number and order of the unique sequences and the recombination sites in the A and D genomes have remained unchanged and constant in each genome and that the increase in genome size is due to increase in repetitive DNA. Recent studies on the renaturation rates of denatured DNA of the A and D genomes show that the A genome chromosomes do indeed have greater amounts of repetitive DNA (Wilson 1974). It has been suggested that one of the possible functions of repetitive DNA is in the regulation of chromosome pairing (Britten 1972; Stack and Clark 1973). Heterochromatin has been identified as repeated sequences of DNA (Yunis and Yasmineh 1970; Yasmineh and Yunis 1971; Arrighi and Hsu 1971; Dolfini 1974; Sanchez and Yunis 1974). Studies by Shaw (1971) and Fontana and Vickery (1974) show that the size and distribution of heterochromatic segments (rep DNA) have a definite influence in the overall control and regulation of chiasma formation. It is apparent that the above functional roles attributed to repetitive DNA are wholly compatible with the findings in *Gossypium* and can be invoked to explain its chromosome pairing behavior. Our working hypothesis is that at the tetraploid level, the regulatory activity of the repetitive DNA would be most effective and the predominating force in controlling regular homologous bivalent formations in *Gossypium*. Thus, the moment the tetraploid level was attained, the roles exercised by the repetitive DNA in structurally differentiating homoeologs and in regulating pairing and chiasma formation would initiate an immediate shift to strict bivalent formation of homologous chromosomes.

In conclusion, we believe that the data in *Gossypium* do not suggest the existence of a gene control pairing mechanism in their natural allotetraploids, but that they do suggest an alternative, non-gene-controlled mechanism as discussed above. Is it possible that there are at least two alternative systems for regulating homologous pairing in allopolyploids combining closely related genomes? The following discussion points out this possibility.

Gene control mechanisms promoting strictly homologous chromosome pairing exists in the allopolyploids of *Triticum* (Sears and Okamoto 1958; Riley and Chapman 1958; Riley, Chapman and Kimber 1959; Feldman 1966; Riley et al. 1966; Kempanna and Riley 1962; Mello-Sampayo 1968; Driscoll 1972) and of *Avena* (Gauthier and McGinnis 1968; Rajhathy and Thomas 1972; Ladizinsky 1973). It is evident that these allopolyploids evolved because of the selection for a genetically controlled mechanism regulating strictly homologous pairing. It is also evident that the allopolyploid of *Gossypium* was successful in its origin and establishment without the requirement of a gene controlled system.

There is one noticeable difference in the two systems. The chromosomes of each of the multiple genomes of *Triticum* and *Avena* are virtually of the same size and morphologically identical, while the two genomes in the alloploid *Gossypium* are markedly different in size and, due to quantitative differences in repetitive DNA, morphologically different. This may be the key to why a gene controlled pairing mechanism is needed in one but not in the other. The difference as proposed here needs further exploration within other polyploid forms by determining the relationships of their genome chromosome size and the system controlling regular bivalent pairing. If a behavior analogous to that in *Gossypium* could be demonstrated in other alloploids with genomes of different sizes, this would provide strong support for the two kinds of pairing control systems.

The final indisputable proof in our case, however, would come from the acquisition and study of a com-

plete set of aneuploids in the allotetraploids similar to those in hexaploid wheat which firmly settled the situation in that species. The monosomic stocks obtained for several chromosomes in cotton have not produced any nullisomic individuals. However, the recovery, though very rare, of two and possibly three ditelocentrics may eventually provide the proper source material to settle the issue.

The one question surely to arise is why the pairing of homoeologs in the $(AD)_1$ haploids and the $A_2D_5$ hybrid differ greatly, and significantly, at MI but not at pachytene. The functional basis of this difference is not known, but clues as to the possible causes may be provided by the reviews on chromosome pairing by Moens (1972) and on chromosome pairing and chiasma formation by Riley and Law (1965). It is generally agreed, and apparent in the latter review, that pairing and chiasma formation in a species are regulated by the integrated operation of a balanced complex of many genes and by the structural organization of the chromosome, which is determined in part by such factors as repetitive DNA, associated histones, and proteins.

Even though pachytene pairing was noted to be more intimate in $A_2D_5$ than in haploids, the two did not differ in the frequency of paired members at this stage. Thus, it seems that the largest component contributing to their observed difference is the modification of chiasma formation. Since little is known of the detailed biological processes that regulate pairing and chiasma formation, we can only give a rather simple, speculative explanation of the possible cause.

It can be rightly assumed that each of the A and D species consisted of a balanced complex of genes and chromosome phenotypes controlling meiotic pairing and chiasma formation that was selected to operate most efficiently at the diploid level. Following the formation of the amphidiploid, diploidization, under the influence of natural selection, would provide a means of returning to, or reselecting for, a system conditioned to operate most efficiently at the tetraploid level. It is assumed that in the process of diploidization a number of subtle quantitative and qualitative genetic changes were selected within the two controlling diploid systems, which established a control system with genetic activity and regulation divided or partitioned between the two genomes. In this particular case selection would be imposed primarily on the system regulating chiasma formation. It is assumed that in haploids of the natural allotetraploids the system would be highly ineffectual in consummating the exchange of DNA strands between homoeologs, resulting in a very low frequency of bivalents at MI.

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