

COLCHICINE-PRODUCED POLYPLOIDS IN *Gossypium*

II. OLD WORLD TRIPLOID HYBRIDS

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(With Four Text-figures)

I. INTRODUCTION

In a previous paper (Stephens, 1942) the compatibility of a colchicine-produced tetraploid Asiatic cotton (*Gossypium arboreum* var. *neglectum* Watt, $4n=52$) with various wild diploid ($2n=26$) cottons was described. The data showed among other things that although diploid *arboreum* failed to set viable seeds when pollinated by the American species, *G. armourianum* Kearney and *G. raimondii* Ulb. or by the Australian species *G. sturtii* F. Muell., yet tetraploid *arboreum* set viable seed readily with pollen from the same male parents. To explain this phenomenon a hypothesis was suggested which was based on the endosperm/zygote genom balance in the hybrid seed. According to this hypothesis, a single genom from American or Australian diploid species is roughly equivalent to two Asiatic genoms in producing the 3 : 2 endosperm/zygote ratio necessary for viable seed production. The three triploid hybrids obtained from these crosses were examined cytologically, since their meiotic behaviour provided useful information of the homologies existing between Asiatic, American and Australian chromosomes. Denoting genoms by letters, the constitution of the triploids was AAA', the two A sets being derived from the tetraploid *arboreum* parent. If A and A' were nearly homologous, A chromosomes would pair frequently with A' chromosomes at meiosis and a situation comparable with that existing in Skovsted's autotriploid (AAA) in which trivalents and higher polyvalents were frequent (Skovsted, 1933) should be obtained. On the other hand, if homologies between A and A' genoms were low the A' genom should be present as univalents. Actually it was found that more than 90% of the A' chromosomes remained unpaired (i.e. the triploids behaved as allotriploids), and it was therefore concluded that homologies between A and A' genoms were low. The data as a whole were in agreement with previous taxonomic and cytological findings (Skovsted, 1937; Hutchinson & Ghose, 1937; Harland, 1939; Beasley, 1942) in showing that diploid species of *Gossypium* fall into at least three well defined major groups—Old World (including Asiatic), American and Australian diploids.

In the present paper an attempt has been made to apply similar methods of analysis to a single group, viz. the Old World diploids. All the known Old World species were examined: *G. arboreum* L., *G. herbaceum* L., *G. anomalum* Wawra. & Peyr. and *G. stocksii* Mast. Unfortunately, the cytological portion of the data is incomplete, since one triploid (F_1 tetraploid *arboreum* \times *herbaceum*) could not be synthesized and another (F_1 tetraploid *arboreum* \times *stocksii*) was a weak unthrifty plant from which only a few flower buds could be obtained for meiotic studies.

II. COMPATIBILITY OF DIPLOID AND TETRAPLOID ARBOREUM*
 WITH OTHER OLD WORLD SPECIES

Diploid *arboresum* crosses readily with other Old World species giving viable F_1 seeds. Its hybrid with *herbaceum* is quite fertile, that with *anomalum* partly fertile (Silow, 1941), and that with *stocksii* quite sterile. On the balance hypothesis of compatibility (Stephens, 1942) the genoms of the four species should be roughly equivalent giving an approximation to the normal 3 : 2 endosperm/zygote ratio as a result of cross-fertilization. It would then be expected that tetraploid *arboresum* crossed by the same diploid species would give unbalanced endosperm/zygote ratios approximating 5 : 3 where the genoms were closely equivalent. The percentage of viable triploid seeds obtained should therefore be inversely proportional to the degree of genom equivalence and in all intra-Old World crosses should be low.

Data given in Table 1 show that this hypothesis is only supported by three out of the four combinations tested. Diploid *arboresum* selfed gave complete compatibility but

Table 1. Cross compatibilities of diploid and tetraploid *arboresum* with other Old World species

| | Pollinations | Percentage bolls set | Percentage fertilized ovules per boll | Percentage viability of fertilized ovules |
|--------------------------|--------------|---------------------------------|---|---|
| | | Diploid <i>arboresum</i> (♀) | | |
| ♂ <i>arboresum</i> (N14) | — | — | 91 | 100 |
| ♂ <i>herbaceum</i> (HTS) | — | — | 95 | 100 |
| ♂ <i>anomalum</i> | 24 | 83 | 95 | 98 |
| ♂ <i>stocksii</i> | 18 | 67 | 50 | 97 |
| | | Tetraploid <i>arboresum</i> (♀) | | |
| ♂ <i>arboresum</i> (N14) | 23 | 0 | * | — |
| ♂ <i>herbaceum</i> (HTS) | 15 | 0 | * | — |
| ♂ <i>anomalum</i> | 26 | 50 | 51 | 74 |
| ♂ <i>stocksii</i> | 25 | 24 | 28 | 26 |

* In tetraploid \times *arboresum* and tetraploid \times *herbaceum* the bolls dropped within 8 and within 21 days respectively. In the case of the *herbaceum* cross the seeds showed radicles and cotyledons clearly differentiated. Attempts are now being made to culture them *in vitro*. Seeds obtained from the *arboresum* cross enlarged considerably and lint was well developed so that it was practically certain that fertilization had taken place, as in the *herbaceum* cross.

tetraploid \times diploid *arboresum* failed to set seed. Similarly, *G. herbaceum*, which is fully compatible with diploid *arboresum* giving a fully fertile F_1 , failed completely to set viable seed with tetraploid *arboresum*. *G. stocksii*, which is highly compatible with diploid *arboresum* but which gives an unthrifty and completely sterile F_1 hybrid, gave a low proportion of viable seeds with tetraploid *arboresum*. It would be expected on this basis that *anomalum*, which is almost fully compatible with diploid *arboresum* giving a vigorous and partly fertile F_1 hybrid, would occupy an intermediate position, i.e. the cross tetraploid *arboresum* \times *anomalum* should give a lower proportion of viable seeds than the cross tetraploid *arboresum* \times *stocksii*. Actually, as shown in Table 1, *anomalum* is highly compatible both with diploid and tetraploid *arboresum*—a situation which cannot be explained on a 'balance' hypothesis. It is clear that compatibility relations in *Gossypium* species are more complex than the writer formerly supposed, and a general survey of the chief factors involved will be presented in a later section of this paper.

* As in the earlier paper, the variety used was N14 (*G. arboresum* var. *neglectum*) and its colchicine-produced tetraploid.

III. MALE MEIOSIS IN OLD WORLD TRIPLOIDS

According to Skovsted (1933) the Asiatic triploid which he examined cytologically had 26 pure *arboresum* chromosomes and 13 derived from an *arboresum* × *herbaceum* hybrid. Meiotic studies showed that it behaved as an autotriploid except for the additional complication that higher polyvalents were produced, probably indicating that *arboresum* and *herbaceum* species are themselves secondary polyploids.* Consequently it was of interest to discover whether the triploid hybrids, F_1 tetraploid *arboresum* × *anomalum* and F_1 tetraploid *arboresum* × *stocksii* would also behave as autotriploids, or whether their meiotic behaviour would more resemble that of the allotriploid (Asiatic × American and Asiatic × Australian) hybrids (Stephens, 1942). Buds were fixed in a warm chromacetic mixture and stained in 1 % acetic orcein.

Metaphase I

In the *stocksii* hybrid, owing to the small number of flower buds available for study, no 1st metaphase plates which could be analysed completely were observed, but five plates were seen in which thirteen univalents could be counted. If these constituted a random sample, allotriploid rather than autotriploid meiotic behaviour was indicated. In the *anomalum* hybrid a different situation existed. Out of twenty 1st metaphase plates examined, eighteen contained trivalents. The degree of chromosome pairing is compared below with other triploid hybrids studied:

| | No. of plates examined | Average no. of | | Auto- ↓ Allo- |
|--|------------------------|----------------|-------------|---------------------|
| | | Trivalents | Polyvalents | |
| Skovsted's autotriploid | 20 | 6.0 | 1.6 | ↑ |
| F_1 tetraploid <i>arboresum</i> × <i>anomalum</i> | 20 | 1.8 | 0.1 | |
| F_1 tetraploid <i>arboresum</i> × <i>sturtii</i> | 20 | 0.9 | 0.1 | |
| F_1 tetraploid <i>arboresum</i> × <i>raimondii</i> | 25 | 0.4 | 0.0 | |
| F_1 tetraploid <i>arboresum</i> × <i>armourianum</i> | 15 | 0.0 | 0.0 | |

It is clear that chromosome pairing in the *anomalum* hybrid approaches the autotriploid type more closely than in other triploid hybrids studied. Even so, the number of trivalents produced is considerably lower than might be expected when it is remembered that diploid *anomalum*/Asiatic hybrids are partly fertile (Silow, 1941). First metaphase plates are shown in Figs. 1, 2.

Anaphase I—Metaphase II

Skovsted found that in his Asiatic autotriploid first anaphase was almost invariably normal. Univalent division did not occur and 95 % of the second metaphase plates showed the full complement of 39 chromosomes. Laggard univalents lying in the cytoplasm were very exceptional. The present writer (1942) found more variable behaviour in Asiatic × American and Asiatic × Australian triploids. Here many of the non-Asiatic chromosomes were not included on the spindle at metaphase I, and at metaphase II they were usually seen scattered in the cytoplasm. Occasionally univalents divided at anaphase I so that more than 39 chromosomes were found at 2nd metaphase. On the other hand, some pollen mother cells showed deficient 2nd metaphase plates showing that chromosome elimination had occurred during the short interphase.

* Considering the cytological aspect alone, Beasley (1942) rightly points out that high associations in polyploids could be due to duplications and not necessarily to secondary polyploidy. This ignores the fact that related genera have multiples or combinations of basic chromosome numbers of 5, 6 and 7 (Skovsted, 1935c). In *Abelmoschus* multiples of 6 and 13 co-exist. '*Gossypium kirkii* Mast'—separated by Skovsted as *Gossypiocides kirkii*—and the related *brevilamatum* have haploid chromosome numbers of 12.

In both *stocksii* and *anomalum* hybrids laggard univalents were common, as indicated by the following figures:

| | No. of univalents scattered in cytoplasm at metaphase II | | | | | | | | Total P.M.C.'s | |
|---|--|----|----|----|----|---|---|---|----------------|-----|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | 8 |
| F_1 tetraploid <i>arboreum</i> \times <i>stocksii</i> | 31 | 20 | 13 | 12 | 6 | 9 | 2 | 2 | — | 100 |
| F_1 tetraploid <i>arboreum</i> \times <i>anomalum</i> | 31 | 21 | 15 | 9 | 12 | 6 | 2 | 2 | 2 | 100 |

In both hybrids therefore only 30 % of the pollen mother cells were free from lagging univalents. The *anomalum* hybrid was examined in greater detail and a normal comple-



Fig. 1.

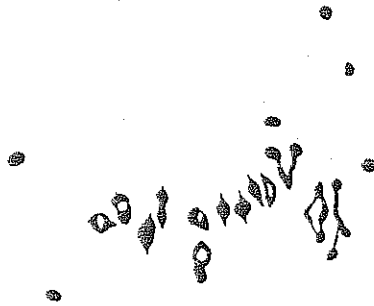


Fig. 2.

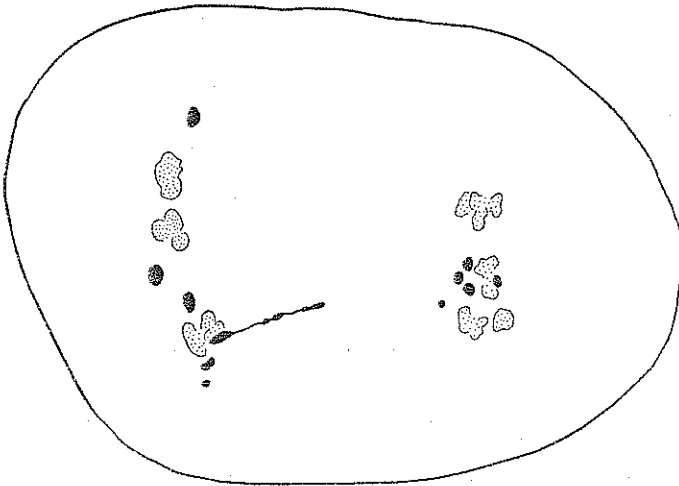


Fig. 3.

Figs. 1-3. Meiosis in the triploid hybrid, tetraploid *arboreum* \times *anomalum*. 1. 1st metaphase showing 4 III's, 9 II's, 9 I's ($\times 3300$). 2. 1st metaphase showing 1 IV, 3 III's, 9 II's, 8 I's ($\times 3300$). 3. Broken bridge at 1st anaphase ($\times 2700$).

ment of 39 chromosomes was found in the majority of the 2nd metaphase plates counted. Univalents divided at anaphase I in about 25 % of the pollen mother cells, but no deficient plates were found. Of special interest is the fact that 5-10 % of 1st anaphase divisions observed in the *anomalum* hybrid showed broken bridges (Fig. 3). These have not been observed previously in triploid hybrids by the writer, but their presence confirms Beasley's

statement (1942) that *anomalum* differs structurally from *arboreum* and *herbaceum*. Beasley considers that at least four structural differences are involved, but the writer has only found convincing evidence of one bridge per pollen mother cell in his own material. It is possible that this apparent discrepancy can be explained by the fact that chiasma formation within relatively inverted segments will be expected to occur more frequently in diploid (AA') than in triploid (AAA') hybrids. According to Skovsted (1937) and Beasley (1942) the F_1 *arboreum* × *anomalum* gives an average of 2.2 univalents, so that, on the average, chiasma formation is only precluded in two *anomalum* chromosomes per pollen mother cell. In the triploid hybrid an average of 10–12 univalents occur. On the assumption that these are *anomalum* chromosomes any relative inversions which they carry will not be detected during meiosis.

Anaphase II—microspore formation

In Skovsted's autotriploid the second division was quite normal, and almost invariably normal tetrads with four microspores were formed. As rare exceptions dyads and hexads were produced. In the allotriploids examined previously by the writer, irregularities occurred, since laggard univalents from the first division set up supernumerary spindles, and abnormal 'tetrads' with varying numbers of microspores were frequent.

The *stocksii* and *anomalum* hybrids showed marked differences in microspore formation. Although, as has been seen above, pollen mother cells in these two hybrids had similar proportions of laggards at 2nd metaphase, these persisted and set up supernumerary spindles in the *stocksii* hybrid but showed a strong tendency to be eliminated at 2nd anaphase in the *anomalum* hybrid. Consequently the latter produced a much higher proportion of normal tetrads than did the former hybrid:

| | Percentage normal tetrads* |
|---|-------------------------------|
| Skovsted's autotriploid | 95 |
| F_1 tetraploid <i>arboreum</i> × <i>anomalum</i> | 81 |
| F_1 tetraploid <i>arboreum</i> × <i>stocksii</i> | 54 |
| F_1 tetraploid <i>arboreum</i> × <i>sturtii</i> | 45 |
| F_1 tetraploid <i>arboreum</i> × <i>raimondii</i> | 85 |
| F_1 tetraploid <i>arboreum</i> × <i>armourianum</i> | 87 |

In the *anomalum* hybrid one 2nd anaphase plate with a bridge was seen (Fig. 4). This could have arisen from the formation of two chiasmata—one within relatively inverted segments and the other proximal to the inversion (Darlington, 1937). It may be concluded from these meiotic studies as a whole, that the *anomalum* hybrid resembles Skovsted's autotriploid much more closely cytologically than any of the other triploids studied but that structural differentiation between Asiatic and *anomalum* genomes is greater than has formerly been supposed. The evidence from the *stocksii* hybrid, scanty though it is, indicates that *stocksii* is distinct cytologically from the other Old World species and suggests the possibility that it may not be more closely related to them than is the Australian species *G. sturtii*, since the meiotic divisions of the *stocksii* and *sturtii* hybrids show a close similarity. This conclusion is in harmony with the findings of Skovsted (1937), Abraham (1940) and Beasley (1942), working with diploid *stocksii* × *arboreum* hybrids.

* Minimum of 200 counted.

IV. GENERAL CONCLUSIONS

(a) *Some aspects of interspecific incompatibility in diploid cottons*

An attempt has been made in Table 2 to summarize the known compatibility relations of diploid ($n=13$) cottons. Data have been compiled from Skovsted (1935*a, b*, 1937), Hutchinson (1939), Harland & Atteck (1931), Boza & Madoo (1941) and unpublished data of the writer.

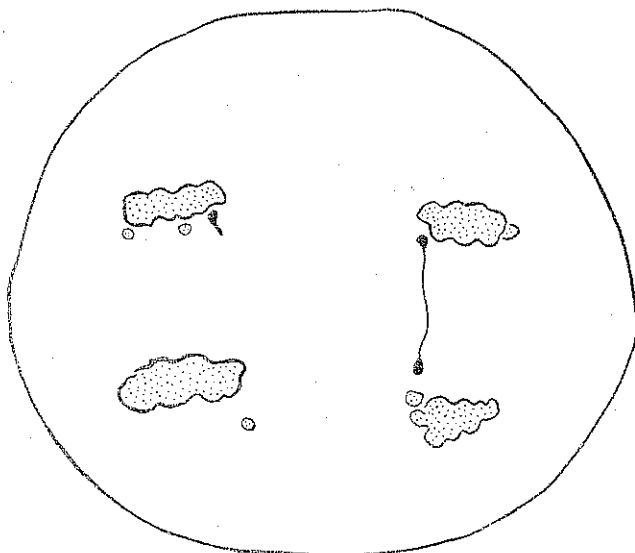


Fig. 4. Meiosis in the triploid hybrid, tetraploid *arboreum* × *anomatum*. Bridge at 2nd anaphase (× 2700).

Table 2. *Compatibility relationships in diploid species of Gossypium*

| | American (♂) | | | | | Old World (♂) | | | | |
|--------------------|-----------------|--------------------|---------------|------------------|-------------------|----------------|-----------------|-----------------|------------------|-----------------|
| | <i>thurberi</i> | <i>armourianum</i> | <i>aridum</i> | <i>raimondii</i> | <i>dauidsonii</i> | <i>sturtii</i> | <i>anomatum</i> | <i>arboreum</i> | <i>herbaceum</i> | <i>stocksii</i> |
| American (♀) | | | | | | | | | | |
| <i>thurberi</i> | .. | - | ± | + | | (-) | | | | |
| <i>armourianum</i> | ± | .. | ± | - | | (-) | | | | |
| <i>aridum</i> | | | .. | - | | (-) | | | | |
| <i>raimondii</i> | - | + | | .. | (-) | | | | | |
| <i>dauidsonii</i> | (-) | (-) | (-) | (-) | .. | (+) | | | | |
| <i>sturtii</i> | | + | - | (-) | | (-) | | | | |
| Old World (♀) | | | | | | | | | | |
| <i>anomatum</i> | ± | (-) | (+) | (-) | + | (-) | .. | + | + | + |
| <i>arboreum</i> | ± | (-) | (+) | (+) | (-) | (+) | .. | + | + | + |
| <i>herbaceum</i> | ± | (-) | (+) | (+) | (-) | (+) | + | + | + | + |
| <i>stocksii</i> | (-) | (-) | (-) | - | (-) | (-) | ±* | + | .. | .. |

- Failure of fertilization.

± Very low success of pollination but viable seeds obtained.

+ Viable seeds obtained.

(-) Cross easily but seeds all empty or on germination give inviable seedlings.

(+) Cross easily giving empty seeds and a very low proportion of viable seeds.

* Hybrids died 'after producing about a dozen leaves' (Skovsted, 1935*a*). The writer has failed to repeat this cross.

The chief interest of the table lies in the evidence it provides on the nature of the inter-specific barriers existing (i) within the Old World group, (ii) within the American group, and (iii) between the American and Old World groups. Crosses between Old World species produce viable seeds. Excluding the crosses involving *G. dauidsonii*, it can be seen that crosses between American species also set viable seeds if fertilization is effected. In crosses between American and Old World species, excluding *thurberi* crosses, the situation is quite

different. Here fertilization is readily effected, but the majority of seeds obtained are inviable. Incompatibility is apparently due to some post-fertilization disharmony (cf. Brink & Cooper, 1941).

It may be suggested that failure of fertilization does not necessarily imply fundamental differences between the parental genotypes as a whole. In certain crosses it would appear that simple mechanical difficulties would be sufficient to prevent foreign pollen tubes from reaching the ovules. For instance, *thurberi* pollen tubes would have to travel two to three times the normal distance to reach the ovules of *G. raimondii* quite apart from any chemotactic considerations. Also the flowers of wild species tend to abscise very easily—a tendency which is no doubt enhanced by the handling of the flower during emasculation. It is possible in fact, pending further evidence, to regard these crosses as potentially compatible combinations providing that the mechanical difficulties of getting the male gametes of one parent in contact with the embryo sacs of another can be overcome. Though these difficulties may be partly genetic they do not necessarily represent major genotypic differences. On the other hand, the failure of seed development after successful fertilization suggests fundamental disharmony between the hybrid zygote and the surrounding tissues. Since Silow (1941) found that the inheritance of compatibility with *dauidsonii* was complex in *arborescens/anomalum* hybrids, it seems more likely that the disharmony is a result of gradual differentiation of the parental genotypes than due to specific complementary lethal effects.

Previous work (Stephens, 1942) suggested that the ability to set viable seeds after successful cross-fertilization was associated with the production of a satisfactory genom balance between endosperm and zygote. Table 2 shows that compatibility relations as a whole do fall into two types associated with the chief taxonomic groups, as predicted by the balance hypothesis. The 'aberrant' crossing behaviour of *G. thurberi*, *G. anomalum* and *G. dauidsonii*, however, shows that balance is only one of several mechanisms involved. It is possible that the poor fertilization encountered in Old World \times *thurberi* may be superimposed on the balance mechanism found in parallel Old World \times American crosses. Methods of improving fertilization artificially (Lewis, 1942) should be worth investigation. *G. dauidsonii* produces empty seeds in crosses with all diploid species except *G. anomalum* which suggests a specific mechanism not found in other types. The data presented in Table 1 indicate that *anomalum* hybrids are insensitive to balance, which to some extent may account for the ability of *G. anomalum* to set viable seeds readily when crossed with *G. dauidsonii*—a capacity which is not shared by other Old World species. These anomalies would be more readily understood if it could be assumed that *G. anomalum* and *G. dauidsonii* represent species which have retained affinities both with the American and Old World groups. There is some evidence that this may be true in the case of *G. anomalum* since phenogenetic studies indicate that its leaf shape development (Stephens, *J. Genet.* 46, 1945) is transitional between Asiatic and American types. Skovsted (1937) noted that the chromosomes of *G. anomalum* were smaller than those of *G. arborescens* and *G. herbaceum* though larger than those of American diploids. Recently, an allopolyploid, *arborescens* \times *anomalum* ($2n=52$) has been obtained which crosses readily with New World ($2n=52$) species. One hybrid with *G. hirsutum* was slightly fertile and two viable seedlings were obtained by open pollination. This suggests the possibility of transferring *anomalum* genes to the 'American diploid' genom in New World cottons.

(b) Interspecific affinities within the Old World group

Silow (1944), as a result of extensive genetic analyses of the three Old World species, *G. arboreum*, *G. herbaceum*, and *G. anomalum*, concludes that intervarietal and interspecific distinctions differ in degree only and not in kind. The same conception of a gradual quantitative differentiation during evolution still holds when comparison is transferred from the genetic to the cytological level. The data which have been presented here show that the triploids obtained by crossing tetraploid *arboreum* with a range of diploid species show a continuous range from Skovsted's autotriploid in which an average of only 2-3 chromosomes remained unpaired, to the *armourianum* hybrid (allotriploid) in which pairing between *arboreum* and *armourianum* chromosomes was not found. It is clear that such continuity could be brought about by differential accumulation of small structural changes. The frequency of gross structural changes (e.g. inversions or changes in homology of sufficient magnitude to produce bridges) in interspecific hybrids which was found by Beasley (1942) suggests the likelihood that small structural changes also occur. These cannot be detected cytologically though their presence may be inferred from the restricted chromosome pairing in interspecific hybrids. In crosses between interfertile species they will behave as Mendelian units and hence will be indistinguishable from true gene mutations. Small structural changes would account for the continuity of the process of differentiation which is seen at all levels of speciation.

If this conception of evolution in *Gossypium* is acceptable, there seems to be no reason for denying species rank to the smallest grouping of organisms, which, even when outcrossing is not inhibited, can preserve itself as a discrete unit. Hutchinson (1944) has described a case where two completely interfertile species of *Gossypium* growing as mixtures under cultivation show no signs of losing their respective identities in successive generations. Although interspecific crossing is known to occur, only those plants which resemble their parent species most closely are able to reproduce their kind under conditions of natural selection. It seems reasonable to suppose that by continuation of gradual divergence, species of the lowest order (i.e. constant breeding units) can eventually reach a level of differentiation where intercrossing is no longer possible (cf. Babcock, Stebbins & Jenkins, 1942). Often, however, before this degree of differentiation can be accomplished by gradual quantitative changes *per se* the process is 'prematurely' concluded by the chance occurrence of gross structural changes in the chromosomes which prevent, either partially or completely, gene exchange. *G. arboreum* and *G. anomalum* appear to have reached this level of differentiation, and Silow's work gives good grounds for believing that gross structural changes should be regarded as accessory to, rather than basically responsible for, their speciation.

When, as a result of divergence, gene exchange between species can no longer be effected experimentally, estimates of species affinity become to a great extent speculative and the available evidence is often conflicting. A good example of such a situation is provided by attempts to assess the affinities between the *arboreum-herbaceum-anomalum* group, *G. stocksii* and *G. sturtii*. Apart from differences in chromosome size (Skovsted, 1935*b*), there is no good cytological evidence for including *G. stocksii* in the Old World group and excluding *G. sturtii*. Yet evidence from comparative morphology (Hutchinson & Ghose, 1937; Hutchinson, 1944), geographic distribution, and cross-compatibility (Table 2) seems to indicate that *G. stocksii* is much more closely related to other Old World species

than is *G. sturtii*. None of these sources of evidence can, however, be regarded as conclusive. Although it is likely that chromosome pairing at meiosis gives a reliable estimate of general genotypic affinity, specific genic control of pairing cannot be discounted. Morphological similarities may often be interpreted either as common familial traits or as independent parallel responses to similar environments. Geographical evidence would appear to be of restricted value when (as here) the species represent extremely ancient, geographically isolated types. Only if differentiation of the genetic mechanism controlling seed setting is typical of genotypic differentiation as a whole, can failure to produce species hybrids be regarded as significant. Probably, final judgement is best reserved pending further evidence, and the suggestion may be offered that phenogenetic analysis such as attempted by the writer (Stephens, 1944) may in the future provide one of the few possible lines of attack.

V. SUMMARY

The compatibility of tetraploid *arboreum* with other Old World species, and the cytology of two resulting triploid hybrids is reported. The results obtained, when considered in relation to diploid species of *Gossypium* as a whole, lead to the following conclusions:

(i) Crosses within the Old World group and within the American group may be considered potentially compatible. In such crosses failure to set viable seed is due to failure of fertilization, and this may be determined by relatively simple mechanical difficulties. On the other hand, crosses between Old World and American species are easily obtained but the hybrid zygote nearly always fails to develop. This incompatibility appears to be due to lack of harmony between the development of the zygote and surrounding tissues. The endosperm/zygote balance is one of several mechanisms involved. The nature of the other mechanisms is still obscure.

(ii) The degrees of 1st metaphase pairing found in synthesized triploid hybrids form a quantitative series ranging from Skovsted's Asiatic autotriploid to the allotriploid, tetraploid Asiatic \times *armourianum*. This suggests that the *gradual* quantitative change shown by Silow (1944) to be responsible for speciation in the *arboreum-herbaceum-anomalum* group is a process which has been continued throughout the genus, and that gross structural changes have been superimposed on the basic mechanism.

(iii) The relationship between *G. stocksii* and other Old World species is still obscure, since the cytological evidence conflicts with other sources of information.

(iv) Evidence is presented which suggests that *G. anomalum* may be regarded as a species bridging the Old World and American groups.

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