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Changes in recombination and genetic disturbance on transferring the H_2Lc_2 linkage group from *Gossypium tomentosum* to *G. barbadense* and *G. hirsutum*

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1. INTRODUCTION

In recent years several authors reporting on the genetics of *Gossypium* have demonstrated reduction in recombination values for linkage groups transferred from one species to another. A critical test confirming this occurrence between diploid species was carried out by Phillips (1961). Where chromosomal segments with linked marker genes have been transferred from diploid to tetraploid species reductions in recombination have similarly occurred (see Rhyne, 1958; Giles, 1961). Stephens (1961) studied recombination between supposedly homologous chromosomes of the two tetraploid species *G. barbadense* and *G. hirsutum* and reported a reduction in the yg_2r_2 region from 20·7% in pure *G. hirsutum* to 8·0% in the interspecies hybrid. He further showed that there was a compensatory increase in recombination in the remainder of the chromosome, leaving the total map distance unaltered. Giles (1961) reported compensation in neighbouring loci when he tested a *G. thurberi* segment which had been incorporated into *G. hirsutum*.

This paper is concerned with the study of a chromosomal segment carrying two linked loci, H_2 (*pilose*) and Lc_2 (*brown lint*) transferred from the wild Hawaian tetraploid *G. tomentosum* to each of the tetraploids *G. barbadense* and *G. hirsutum* race *punctatum*.

Detailed records of families through seven successive backcrosses were kept. The changes which occurred both in recombination and in disturbance to single factor segregation ratios are described in relation to the steady reduction in the amount of *tomentosum* chromatin in the hybrid plants of each backcross generation.

2. PREVIOUS WORK

The linkage between H_2 and *brown lint* was first reported by Hutchinson (1946). It is now known that this group is located in the A subgenome of the (AD) genome of the tetraploid cottons (Gerstal & Phillips, 1958; Endrizzi, 1963).

Stephens (1955), investigating the linkage relationship of H_2 and *brown lint*

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(for which he used the symbol Lc_2), tested three brown linted stocks. 'Higginbotham Brown' and 'Brymer Brown' were both *G. hirsutum* Upland non-pilose stocks. The third variety was also *G. hirsutum* but with the segment H_2Lc_2 introduced from *G. tomentosum*. The extent of the backcrossing to *G. hirsutum* following the transfer was not indicated.

A summary of the values obtained by Stephens is given below:

Backcrosses	Parent stock	Recombination percentage	No. of plants
(a)	Higginbotham	11·8	34
(b)	Brymer	17·9	39
(c)	*G. hirsutum* with *G. tomentosum* segment	4·8	63
F_2	F_2 of (c)	15·2	53

Stephens gave a joint estimate of 10·3 % recombination for the backcross data but comments upon the fewer crossovers in the *tomentosum* backcross and the poor agreement of the F_2 value of 15·2 %.

Estimates of linkage can be made from Hutchinson's (1946) families. *G. tomentosum* was crossed to each of the species *G. barbadense* and *G. hirsutum* and first backcross families were obtained. With *G. barbadense* as the recurrent parent a linkage value of 20·7 % was found and with *G. hirsutum* as the recurrent parent the value was 20·6 %.

The families on which these various estimates are based are small and few in number and leave considerable doubt as to the true value. Furthermore the low recombination value of 4·8 % which Stephens obtained for the *tomentosum* segment in *G. hirsutum* is similar in kind to more recent findings of reduced recombination in interspecific transferences.

Endrizzi & Kohel (1966) investigated the linkage group H_2Lc_2 using a marked telosomic chromosome and established the relationship of H_2 and *Lc* to the centromere of chromosome 6. The map distance between the two loci was found to be 22·1 units and the centromere, which is located between them, was 4 units from H_2. *Lc* is given no numeral subscript because, citing Dr B. A. Waddle currently investigating the *brown lint* genes, there is now evidence that those in 'Brymer Brown' and 'Higginbotham Brown', although located on the same chromosome, are not allelic. Their data refer to test material carrying 'Brymer Brown' and the value of 22·1 % obtained is in reasonable correspondence with Stephen 'Brymer Brown' value of 17·9 % but is distinctly different from 11·8 % for 'Higginbotham Brown'.

Pending the clarification of the identity of the *tomentosum brown lint* allele the gene symbol Lc_2 will be used in this paper in conformity with previous descriptions.

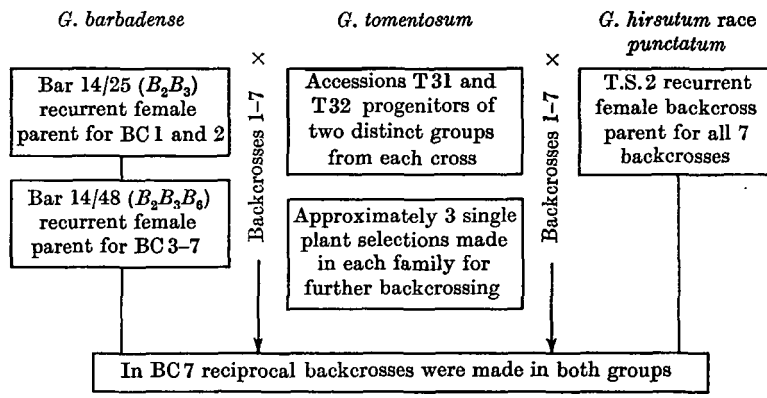
3. MATERIALS AND METHODS

Two accessions of *G. tomentosum* (H_2Lc_2) were used, T 31 and T 32. One plant from each was used as the male parent in the initial cross to each of the tetraploid cottons *G. barbadense* and *G. hirsutum* race *punctatum*

Two strains of *G. barbadense* (h_2lc_2) were used. They are similar in origin and are bacterial-blight-resistant derivatives of the long staple commercial crop of the Sudan which is known in general as Sakel cotton. The first two backcrosses were made to Bar 14/25 (resistant genes B_2B_3) and the remaining backcrosses to Bar 14/48 (resistant genes $B_2B_3B_6$). B_2 and B_3 were transferred to Sakel from *G. hirsutum* and B_6 from *G. arboreum*. A minimum of 15 generations of backcrossing to Sakel types have occurred since the transfer, together with many self-bred generations with rigorous selection for Sakel cropping characteristics. They are identical with Sakel in appearance and are very uniform for plant type. The *punctatum* (h_2lc_2) parent, type T.S. 2, is a completely glabrous cotton collected by H. E. King near Lake Chad in N. Nigeria. This parent is also very uniform. Both species were used as bulk female parents in the backcrosses to facilitate the harvesting of large amounts of seed.

The programme of backcrossing is set out schematically in Table 1.

Table 1



When homozygous on a *barbadense* background the gene Lc_2 confers a medium brown colour to the lint. In the heterozygous state (Lc_2lc_2) the colour expression is lighter but distinct from the creamy white of Sakel. The brilliant white of the *punctatum* lint made the distinction between classes simple. During the later backcrosses the recording was carried out in the field jointly by my colleague Mr R. Gunn and myself; all cross-overs were double-checked. Sakel is a glabrescent cotton with a few sparse hairs on the lower leaf lamina. T.S. 2 is a glabrous strain and *G. tomentosum* is densely pilose. The pilose gene H_2 is strongly expressed in the heterozygous condition H_2h_2 and is therefore easily distinguished from h_2h_2 in the backcross progenies.

Following the initial cross between *G. tomentosum* and the two tetraploid species, pilose plants were taken at random in each generation (usually three plants from a family) for further backcrossing. At the time of selection the lint colour was not known and therefore the family recombination value was not known either. Selections were also made without reference to earlier recombination values of parental progenies. Thus there was no bias in favour of low or high linkage inheritance.

The single factor ratios for both lint and hair genes are disturbed to a greater of lesser extent throughout. Recombination percentages have for this reason been calculated by the product ratio method.

The volume of data is too great to permit detailed presentation. There was no evidence to suggest that the two accessions of *punctatum*, T 31 and T 32 differed genetically with respect to the affects of the segment H_2Lc_2, nor was there any reason to regard any family within a generation as differing fundamentally from any other. Wide differences in recombination percentages were recorded for each generation but there was no evidence that a low value in one generation led to a low value in the next and similarly for high values. For simplification of presentation therefore the backcross data for each generation have been pooled. The distortion in the single factor segregations which is so marked in the *barbadense* data will be shown to be associated with the H_2 locus or a close segment and not Lc_2 and for this reason only the segregation of H_2, h_2 is given in the tables.

4. RESULTS

The first backcrosses to each of the recurrent species *G. barbadense* and *G. hirsutum* race *punctatum* were clearly genetically disturbed. Many plants were stunted, unthrifty and infertile. The second backcross still showed disturbance but to a lesser extent. By the third backcross reasonable uniformity of progenies was apparent, the *punctatum* material closely resembling the recurrent parent. The single factor segregation, H_2, h_2 was very disturbed in both lines of transference, but by BC 4 in the *punctatum* material was in conformity with a 1 : 1 ratio. Disturbance continued in the *barbadense* backcrosses.

The total data for each backcross generation have been pooled and are given in Tables 2 and 4 for the transferences to *G. barbadense* and *G. hirsutum* race *punctatum* respectively.

(*a*) *Disturbance to the segregation of* H_2, h_2 *in backcrosses to* Gossypium barbadense *using heterozygous males*

In each backcross generation the heterozygous selections (H_2Lc_2/h_2lc_2) were used as male parents and the recurrent *barbadense* (h_2lc_2/h_2lc_2) strain as bulk female parents. The segregation of H_2, h_2 in all generations from BC 3 to BC 7 gave ratios of 1 pilose to 2 glabrescent (Table 2). Because the expected ratio was 1 : 1 there was an observed deficiency of 50 % of the pilose plants. In a few families even greater shortage of pilose plants was recorded but progenies derived from

such families gave 1:2 ratios. In BC 7 the selected heterozygotes were used both as male and female and sibling progenies were raised. When the genotype of the female parent was H_2Lc_2/h_2lc_2 there was a normal 1:1 backcross ratio (Table 2 BC 7b).

Table 2. *Transference of* H_2Lc_2 *to* Gossypium barbadense

	Backcross generation					
	3	4	5	6	7a	7b
No. of families ...	5	11	31	24	9	9
Recombinants						
H_2Lc_2	276	681	1746	1031	555	253
H_2lc_2	48	51	125	98	34	51
h_2Lc_2	84	110	169	175	57	75
h_2lc_2	544	1309	3370	2129	1276	239
Recombination percentage	16·4	7·9	6·0	8·8	5·0	20·1
H_2	324	732	1871	1129	589	304
h_2	628	1419	3539	2304	1333	314
χ_1^2 (1:2)	0·2	0·5	3·8	0·3	6·2	0·2

NOTE. The heterozygote (H_2Lc_2/h_2lc_2) was the male parent in all backcrosses from 2 to 7a and female in 7b.

This result is explicable if, in addition to H_2h_2, there is another independent heterozygous locus segregating, one of the alleles of which, when present with H_2 in the male gamete, is deleterious in action. Thus there are four genotypic classes of gametes: two with H_2 and two with h_2. One of the H_2 male gametes fails when the hybrid is the pollen parent, and the observed ratio is 1 hairy:2 glabrescent. The question arises whether this interaction is due to the whole of the introduced *tomentosum* segment H_2Lc_2 or only part of it. In BC 5 six plants which were pilose but lacking brown lint, i.e. genotypically H_2lc_2, were backcrossed to *barbadense* females and five of the six progenies were in excellent agreement with the disturbed 1:2 ratio. The sixth had 86 pilose to 129 glabrescent plants (χ_1^2 (1:2) = 4·30). The total for all families was 416:761 for the same classes (χ_1^2 (1:2) = 2·14). In BC 3, three brown-linted glabrescent plants (h_2Lc_2) were backcrossed to *barbadense* females. The segregation of Lc_2:lc_2 was 62:81, 47:62 and 54:58 with a total of 163:201. The total departs significantly from a 1:1 but the individual families do not. Disturbance is associated with Lc_2 as well as H_2 but is less severe. Later backcrosses show that disturbance at the Lc_2 locus is reduced. It is possible therefore that the cause of the disturbed ratio is not H_2 but rather a part of the *tomentosum* segment between the two loci nearer to H_2 than Lc_2. The cross-over classes in each backcross generation support this contention. In Table 2 the 3rd, 4th and 6th backcrosses are in good agreement with the occurrence of twice as many glabrescent brown (h_2Lc_2) plants as pilose whites (H_2lc_2). The 5th and 7th have rather more pilose whites than expected on this basis. However, the figures show clearly that the loss of segregants is concerned with H_2 or some closely linked locus. For brevity H_2 only will be referred to in the following data and discussion.

The interacting locus, which with H_2 causes gametic malfunction, does so only in the pollen and not in the ovules. It is regarded then as an inhibitor and not as a lethal and will be designated by the symbol I. It is convenient to regard this as a unit gene but there is no proof that it is not a chromosome segment. The alleles at this locus for *G. barbadense* and *G. tomentosum* will be distinguished as I^B and I^T respectively.

The pilose selections made in each backcross are H_2h_2. Half the H_2 pollen is ineffective and therefore the I locus must be heterozygous I^BI^T. The recurrent parent, *barbadense*, is $h_2h_2I^BI^T$. If I^T interacted deleteriously with H_2 in the *barbadense* genetic background all pilose brown plants would be $H_2h_2I^BI^B$, I^T would be eliminated and the 1:1 ratio of pilose to glabrescent restored. This is not the case and the combination H_2I^B is the cause of the failure of the male gametes.

Table 3. F_2*'s of the fifth backcross to* Gossypium barbadense *female*

	Observed			
Families	H_2	h_2	Total	χ_1^2 (2:1)
G189/62	72	31	103	0·48
G190/62	45	18	63	0·64
G191/62	38	27	65	2·00
G192/62	91	37	128	1·13
G193/62	87	49	136	0·44
G194/62	14	7	21	0·00
G195/62	35	13	48	0·84
G196/62	81	33	114	0·99
Totals	463	215	678	0·80

Further evidence in support of this interpretation is found in progenies raised from self-bred hybrid plants. If in such families H_2I^B pollen fails, there will be a loss of four out of the twelve H_2-carrying zygotes and the resulting segregation will be 8 pilose to 4 glabrescent plants, or a ratio of 2:1.

Eight of the H_2Lc_2/h_2lc_2 selections in BC 5 were selfed. Their progenies are given in Table 3 and all are in excellent agreement with the postulated 2:1 ratio. The genotype $H_2h_2I^BI^B$ should occur in the F_2 (h_2I^B pollen and H_2I^B ovule). If this combination were not viable fewer pilose plants should be recovered; this was not detected in the figures.

Knight (1952) transferred H_2 from *G. tomentosum* to Sakel and in his segregating progenies classified his plants as 'hairy' or 'glabrescent'. He gave the combined total segregation of 'hairy' to 'glabrescent' in three first backcross families as 187:199. This is an acceptable 1:1 ratio and agrees with first backcross results in this study where five out of six families also bad good 1:1 ratios and the sixth had an excess of H_2 plants. The total for all families was 173:152 for the same classes. Knight's second backcross was small, 20 'hairy' to 35 'glabrescent' but a shortage of H_2 genotypes is clear. He also gave figures for the 2nd to 5th backcrosses to Sakel females. The first three of these were definitely deficient in the

'hairy' class. The totals for all backcrosses were 108:142. The disturbed ratios discussed in this paper were most clearly marked from the third backcross onwards. Knight crossed three plants from the F_1 of *tomentosum* × *barbadense* with a Sakel strain homozygous for H_1. He was easily able to distinguish between plants carrying H_2 and those from which it was absent. He recorded 93 plants with H_2 and 119 with h_2. There was no serious deficiency of H_2 in this first backcross. He selfed the bulk of the H_2 group and obtained 393 'hairy' plants due either to H_2 or H_1 or a combination of both and 36 'glabrescent' plants. He had demonstrated that H_2 and H_1 were non-allelic but his ratio for 'hairy' to 'glabrescent' was 11:1 and not 15:1 as expected. If the same mechanism causing pollen failure operated in his material as is postulated here then the 2:1 segregation for H_2, h_2 and the 3:1 normal F_2 segregation for H_1, h_1 in his F_2 together would in fact have had an expectation of exactly 11:1 'hairy' to 'glabrescent' plants. Finally, after

Table 4. *Transference of* H_2Lc_2 *to* Gossypium hirsutum *race* punctatum

Backcross generation ...	2	3	4	5	6	7a	7b
No. of families	4	10	11	28	15	12	12
Recombinants							
H_2Lc_2	97	385	999	1388	1303	232	316
H_2lc_2	6	9	18	28	18	4	0
h_2Lc_2	5	12	30	11	8	1	0
h_2lc_2	130	545	1060	1422	1381	261	328
Recombinant percentage	4·9	2·3	2·3	1·2	0·9	0·9	0·0
H_2	103	394	1017	1416	1321	236	316
h_2	135	557	1090	1433	1389	262	328
χ_1^2 (1:1)	4·3	27·9	2·5	0·1	1·7	1·4	0·2

NOTE. The heterozygote (H_2Lc_2/h_2lc_2) was the male parent in all backcrosses from 2 to 7a and female in 7b.

three backcrosses to $H_1H_1h_2h_2$ Sakel in which H_1 became homozygous he selfed $H_1H_1h_2h_2$ plants. These yielded a total of 34 'fully hairy' plants ($H_1H_1H_2H_2$), 103 'less hairy' plants ($H_1H_1h_2h_2$) and 54 with a moderate hair cover, typical of H_1 ($H_1H_1H_2h_2$). The segregation for H_2, h_2 in the absence of the inhibitor I^B would be expected in the ratio of 1:2:1 for the above classes. The total χ^2 for all classes is 5·37 with two degrees of freedom ($P > 0{\cdot}05$). If, however, there is a real loss of hairy genotypes due to the gene I^B, the expected two-gene ratio for the same classes is 2:6:4 and the calculated χ^2 is 2·21 ($P = 0{\cdot}3$). The observed figures are in better agreement with the latter hypothesis and provide further evidence that the inhibitor I^B was present in Knight's material.

(*b*) *Disturbance to the segregation of* H_2, h_2 *in the backcrosses to* Gossypium hirsutum *race* punctatum

In Table 4 data showing the segregation of H_2, h_2 for six generations are given. In BC 2 and 3 a serious shortage of H_2 genotypes is apparent, the observed

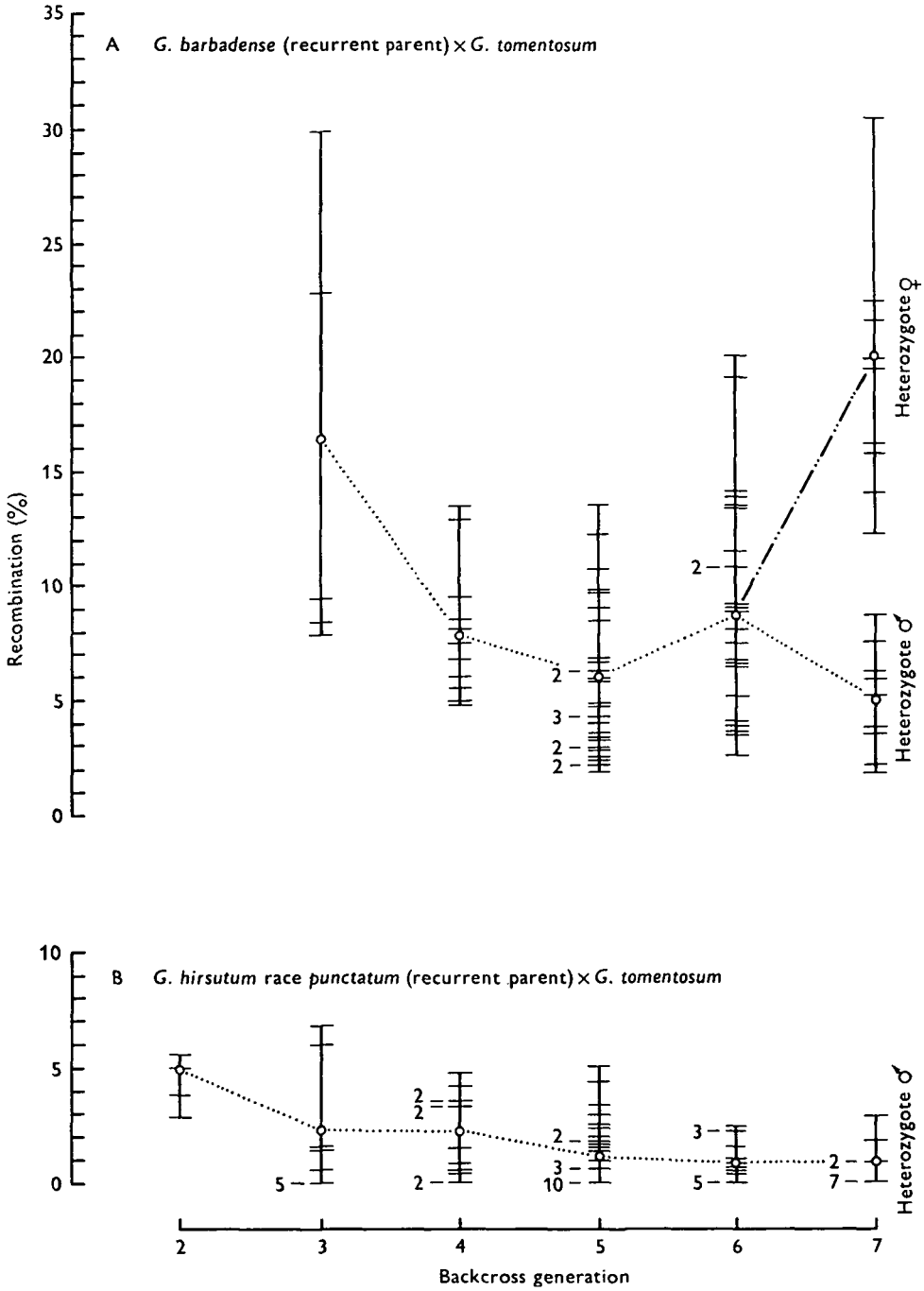


Fig. 1. Inheritance of recombination in the H_2Lc_2 interval in successive backcrosses. The number of progenies with the same recombination value is indicated to the left in each generation. Open circles joined by broken lines are the joint recombination values. Eleven reciprocal backcross progenies (heterozygote female) in BC 7 in B are not indicated. There was no recombination.

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ratios differing significantly from a 1:1 expectation. BC 4–7 are stablized in their agreement with a 1:1 ratio and consistent shortage of H_2 genotypes. This phenomenon will be discussed later as the selective elimination of donor parent genes.

(c) *Reduced recombination with advanced backcrossing*

The outstanding characteristic of the values obtained for the crossing-over percentage in the segment H_2Lc_2 was their extreme variability. This was much more marked in the *barbadense* material. Figure 1 A and B shows graphically the progressive reduction in the values obtained for recombination. A joint estimate of recombination for each backcross generation is plotted and the individual family values are indicated throughout the range. The observed totals for all families are given in Tables 2 and 4 with the joint recombination figures. In the *barbadense* data this starts at a high value of 16·4 % for BC 3 reducing to 5·0 % by BC 7. There was a temporary rise in BC 6 at 8·8 %, the previous BC 5 generation being 6·0 %. In the *punctatum* transfers, values were much lower from the beginning. BC 2 was 4·9 % with a steady reduction to 0·9 % in BC 7. In all these families the heterozygote was the male parent.

In BC 7 all selected hybrid plants in both transference lines were backcrossed reciprocally to their recurrent parents. The pooled data for each interspecific transference are given under BC 7 a and b in each of the Tables 2 and 4. In *barbadense* there was a dramatic restoration of a high recombination value in every family when the heterozygote was female. The values obtained for nine sibling pairs were: 19·4, 8·8; 15·7, 5·8; 16·2, 3·5; 19·9, 3·2; 21·6, 5·2; 30·4, 7·6; 12·3, 3·7; 22·4, 6·3 and 14·1, 1·9. The heterozygous parent is female in the first figure and male in the second figure of each pair. A positive correlation exists between high and low values ($r = 0{\cdot}585$). In *punctatum* the situation differed. Out of ten sibling pairs, in seven both had the value 0·0 and in the remaining three the pairs were 0·0, 0·9; 0·0, 2·9 and 0·0, 1·8. Recombination had virtually ceased, and when found it only occurred where the heterozygote was female, the opposite case from that with *barbadense* material.

Two anomalous values were obtained in small families in the BC 7 of *punctatum*. These were not pooled with the remaining data. The two sibling pairs in which these were recorded are given below:

		H_2Lc_2	H_2lc_2	h_2Lc_2	h_2lc_2	Recombination percentage
T 31	Heterozygous male	13	2	1	6	13·8
	Heterozygous female	27	0	0	33	0·0
T 32	Heterozygous male	9	4	0	14	14·8
	Heterozygous female	29	0	0	25	0·0

These very high values of 13·8 and 14·8 are atypical of the material as a whole and the plants in each family are too few to give confidence. However, one sibling pair came from each of the accession lines T 31 and T 32 and the occurrence cannot be dismissed from the record. It will be noted, however, that crossing-over was confined to the heterozygous male line as in all other BC 7 pairs.

5. DISCUSSION

Depressed vigour, partial sterility and disturbed genetic segregations in progenies from self-bred interspecific hybrids of *Gossypium* are commonly encountered. Harland (1936) proposed that 'multiple gene substitution' within species had created 'modifier complexes' which buffered the larger effects of major genes. The evolution of such systems within species led to imbalance in hybrids. Stephens (1950), on cytological evidence of structural differentiation between homoeologous chromosomes of distinct species, argued that these were more potent causes of genetic breakdown in hybrid progenies and consequently of more importance in determining species differences. In 1961 he reviewed all available evidence, concluding that neither theory was mutually exclusive, both being important in their contribution to the evolution of species within *Gossypium*.

Discoveries of differences in chromosomal architecture continue and a recent analysis of the races of *G. hirsutum* by Endrizzi (1966) has shown that such differences are also to be found at the subspecific level.

No gene or chromosome functions in isolation but is part of the balanced collective action of the genome. Two or more genomes may be combined in a hybrid, and a vigorous plant, often exhibiting heterosis, will result. The first-generation hybrid between *G. barbadense* and *G. hirsutum* is an example of this kind and is extremely vigorous and fertile. Such hybrids may be sterile due to irregularities of meiosis. Structural differences between chromosomes may prevent bivalent formation or lead to unbalanced meiotic products. On the other hand, genetic breakdown will almost certainly occur in the progeny from a highly fertile interspecific F_1. In such F_2 or backcross progenies the balance of the parental genome units is upset.

The present study is limited to a single segment H_2Lc_2 of the wild species *G. tomentosum* transferred to each of the species *G. barbadense* and *G. hirsutum* race *punctatum*. In the hybrid material to which this has led, many of the phenomena referred to above were observed and recorded. The progressive reduction of recombination between transferred linked genes and reciprocal differences of those values in backcrossing are recorded for the first time for *Gossypium*.

The shortage of H_2 plants in the *punctatum* backcrosses has been noted. This was severe in the first two recorded generations and confined to a persistent though small deficiency from then onwards. Stephens (1949) investigated this upset to segregation in single gene ratios where the donor gene was deficient. He described this as the selective elimination of donor parent genes and occurred when interspecific hybrids were backcrossed to the recipient parent; this was unaffected by the direction of the cross. The severity of loss varied according to the locus being studied He also recorded a significant *excess* of one donor allele. In these data families occurred with a significant excess of H_2 genotypes It would seem that usually the introduced gene or segment is at a competitative disadvantage compared with the corresponding segment of the recurrent parent, but that occasionally the advantage lies with the new introgressed segment.

This may not persist due to the breakdown of the gene combination by which it was favoured following further segregation and recombination.

The first *barbadense* backcross in which ten families totalled 284 pilose to 301 glabrescent plants appeared to parallel the behaviours of H_2Lc_2 in *punctatum*, where a 1:1 ratio was distorted by the selective elimination of H_2 genotypes. However, by BC 3 this shortage had increased to 50 % and remained consistently so whenever the heterozygote was the male parent. A simple interaction between H_2Lc_2 and I^B has been postulated to account for the loss of half of the male gametes of the heterozygote. There was no apparent additional shortage of H_2 plants due to selective elimination of the type seen in *punctatum*. It may be argued from this that the *barbadense* chromosome carrying h_2lc_2 is structurally more closely similar to the *tomentosum* H_2Lc_2 chromosome than is the *punctatum* homoeologue.

In the *barbadense* × *tomentosum* hybrid the interaction between the H_2Lc_2 segment from *tomentosum* and the *barbadense* inhibitor I^B always resulted in the non-functioning of the male gametes in which they both occurred. Nevertheless in the first backcross, as was stated above, a near 1:1 ratio was recorded. This was also true of Knight's (1952) first backcross families. In these early backcross generations the amount of *tomentosum* chromatin relative to the contribution of the recurrent backcross parent *barbadense* was high. It is suggested that this may have had the effect of buffering the *tomentosum* H_2Lc_2 segment (which may also have been quite large) that I^B was ineffective.

Reasons have been given for suggesting that H_2 itself may not be the locus concerned in the interaction with I^B. No direct proof is available in these data but in unpublished work of Knight available to the author it is clear that he obtained pilose Sakel progenies with white lint, geotypically H_2lc_2, which in backcrosses to commercial Sakel (h_2lc_2) females gave 1:1 ratios and self-bred H_2h_2 plants from these gave 3:1 ratios. In these H_2lc_2 plants the Lc_2 section of the *tomentosum* segment had been replaced by lc_2 from Sakel (*barbadense*) and it seems likely that the deleterious part of the segment was removed.

The failure of gametes was confined to the pollen. That it was post-meiotic is clear since the products of recombination were evident in the progenies. The point at which failure occurred is not known but the effective functioning of the pollen carrying the H_2 allele required the presence of two distinct *tomentosum* segments on independent chromosomes.

Linked genes transferred from one species to another in *Gossypium* usually undergo a reduction in frequency of recombination compensated for by an increase in crossing-over in other parts of the chromosome (authors cited earlier). This is in accord with the belief that the total recombination per bivalent is genetically determined (Darlington, 1939; Stephens, 1961), but that the site of the exchange is controlled or influenced by the relationship existing between the chromosome pairs. It is apparent from Fig. 1A and B that the linkage of H_2Lc_2 became tighter with each successive backcross to the point, in *punctatum*, where recombination virtually ceased. Thus the gradual elimination of *tomentosum*

chromatin led to the preferential exchange of the homologous sections of the *punctatum* chromosomes carrying H_2Lc_2 or h_2lc_2. However, it would seem that in the case of the *barbadense* transfer this reduction in recombination value only occurred in the heterozygous males where, by BC 7, the joint estimate of recombination was 5·0 %, while the joint estimate for the reciprocal cross was 20·1 % (Table 1, BC 7a and b). This latter figure is in good agreement with those of Stephens (1955), 17·9 % for H_2Lc_2 (Brymer Brown), and Endrizzi & Kohel (1966), 22·1 % for the same segment; these values were obtained in pure *hirsutum* stocks. When Stephens (1955) transferred H_2Lc_2 from *G. tomentosum* to *G. hirsutum* race *latifolium* he obtained a recombination value of 4·8 % after an unstated number of backcrosses to *latifolium*. This is of a similar order to the low values obtained in the present work for the *punctatum* transfer.

The reaction of each of the two species *G. barbadense* and *G. hirsutum* race *punctatum* to the inclusion of the *G. tomentosum* segment H_2Lc_2 is very different in terms of recombination values. In *punctatum* the highest value recorded was 6·8 % in BC 3, falling to a joint estimate in BC 7 of 0·9 %, the heterozygote in all cases being the male parent. When used as female in BC 7 no crossing-over at all was recorded (Table 4, 7b). A similar result had been obtained in other families in BC 6.

On the other hand in the *barbadense* crosses the highest values were obtained when the heterozygote was female; as already stated, the values match other independent estimates. Thus in the egg cells recombination in the H_2Lc_2 region seems to be unaffected, but in the male there is considerable reduction. Each generation showed extreme variation for these values (Fig. 1A). The only factor known to change from generation to generation is the ratio of *barbadense* to *tomentosum* chromatin. Each successive backcross to *barbadense* eliminates some part of the residual *tomentosum* material; selection retaining H_2Lc_2 and any tightly linked chromatin. The lowest recombination figures recorded are in the later backcrosses and it must be assumed that in these progenies the *tomentosum* segment has been reduced to its smallest size and that other *tomentosum* inclusions largely eliminated. It may be postulated that preferential recombination takes place between homologous *barbadense* sections of the chromosome pair in question, to the detriment of crossing over between H_2Lc_2 and h_2lc_2.

Rhyne (1958) also recorded a wide diversity of recombination values when *Gossypium* diploid linkage groups were transferred to *G. hirsutum*. He attributed this in some measure to species and strain genotypic effects, although no statistical significance was found. In the present work no segregation took place into high and low lines, but instead low recombination sometimes occurred in families derived from parental progenies with high frequencies, and these in turn gave high and low values in their derivatives. The lines of descent through succeeding backcrosses form a reticulum, the limits of which is the range in each generation. The mean path of the reticulum, measured by the joint recombination values, follows a definite trend of diminishing values. The selective elimination of H_2 can be attributed to the upset in the functional balance of the cell caused by the

H_2 locus itself or some closely linked part of the introduced chromatin. This can also be invoked to explain variation in recombination values within the H_2Lc_2 segment since no two selected hybrid plants in any backcross generation is likely to be exactly similar in the constitution of the foreign segment. However, it seems equally likely that environmental causes contribute to the observed results. The trend to lower recombination values is accompanied by what appear to be random high and low cross-over percentages. Some support for this may be found in the unexplained temporary increase in recombination value for BC 6. If this was a seasonal effect then the other variability may also be due to external causes. The impression is therefore gained of extreme sensitivity of the *barbadense* genotype to the introduced H_2Lc_2 segment. The reciprocal differences in recombination values suggest that the internal environment of PMC's and EMC's influence the mechanism of crossing-over. Malfunction is more frequently associated with male gametes than with egg cells which are protected and well supplied with all nutrients. Where reduced recombination is only found in the male it may be attributable in some way to the more exacting conditions of development.

In a review of the observations made in this study some tentative conclusions may be reached concerning the evolutionary divergence of the wild tetraploid *tomentosum* and the two cultivated species.

In the first two backcrosses to *punctatum* there was a rapid elimination of the donor parent (*tomentosum*) material and full self-fertility was evident in BC 3. This apparently proceeded more slowly with the backcrossing to *barbadense*. Knight (1945) noted that after two or three backcrosses following an interspecific cross the progenies closely resemble the recurrent parent. Stephens (1949) discussed this in terms of the mechanism of selective elimination and gave reasons for believing that elimination is gametic in the early backcrosses. The most rapid elimination of donor parent chromosomes can be expected where the original cross was made between species which have undergone the greatest evolutionary change in their chromosomal architecture. Thus in the *punctatum* material an early and rapid elimination of *tomentosum* material took place. Greater disturbance was observed in the *barbadense* backcrosses where there was possibly more recombinations which preserved greater hybridity than was the case with *punctatum*. This implies that there is more similarity between *barbadense* and *tomentosum* chromosomes than between those of the latter and *punctatum*. The interaction of I^B and H_2Lc_2 in *barbadense* is the incompatibility of one gene or one chromosome from each species, whereas the selective elimination of H_2 is probably due to imbalance between *tomentosum* and the recipient species. This is unmistakable in *punctatum* but not detectable in *barbadense*. There would appear to be evidence here of greater chromosomal differences between *tomentosum* and *punctatum*. If the recombination values obtained when the heterozygote is female are accepted in the *barbadense* cross as being undisturbed then these, by virtue of their close agreement with values within pure species, suggest a similarity of the chromosomes in question in each of the species *barbadense* and *tomentosum*, whereas the large reduction in recombination accompanying the transference to *punctatum* must

indicate inter-species chromosomal differentiation. The sum total of the evidence therefore points to less evolutionary divergence between *tomentosum* and *barbadense* than between *tomentosum* and *punctatum*.

SUMMARY

The H_2Lc_2 linkage region from *G. tomentosum* was transferred through seven successive backcrosses to *G. barbadense* and *G. hirsutum* race *punctatum* in which these species were used as bulk female recurrent parents. In the last backcross, BC 7, reciprocal crosses were also made. Recombination values diminished with progressive backcrossing. Other workers have shown that values of the order of 20·0 % within the pure species were to be expected. In BC 7 joint estimates for *punctatum* were 0·9 % and 0·0 % as female and male respectively; the corresponding estimates for *barbadense* were 5·0 % and 20·1 %.

Selective elimination of the donor gene H_2 on transference to *punctatum* was observed in all backcrosses, causing disturbance to the expected 1:1 backcross ratio. Disturbance of a different order was recorded for the segment H_2Lc_2 transferred to *barbadense*. When the heterozygote H_2Lc_2/h_2lc_2 was male and *barbadense* (h_2lc_2/h_2lc_2) female 50 % of the H_2Lc_2 gametes were lost and a 1:2 backcross ratio was observed instead of the expected 1:1. The 1:1 ratio was restored when the heterozygote was the female parent. The lethal interaction of the H_2Lc_2 *tomentosum* segment with an inhibitor I^B from *barbadense* was postulated.

Differences in the reaction of the genotypes of *G. barbadense* and *G. hirsutum* race *punctatum* to the introgression of H_2Lc_2 from *G. tomentosum* were discussed in terms of evolutionary divergence of the species.

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