ENHANCING LINKAGE-BLOCK BREAKUP FOLLOWING
INTERSPECIFIC HYBRIDIZATION AND BACKCROSS
TRANSFERENCE OF GENES IN *GOSSYPIMUM HIRSUTUM* L.¹

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STEPHENS (1950) stated that blocks of linked characters (sic) rather than individual genes are transferred from interspecific hybrids by repeated backcrossing with *Gossypium hirsutum*. According to HANSON (1959a), in species having normal chromosomes of short map length, the rate of breakup in a linkage block is exceptionally slow in a backcrossing program. For example, he predicts that at the end of backcross 20 in a species having a chromosome of 0.50 map length, the heterozygous segment containing the desirable gene should have a 0.10 map length. In a wide species cross, according to HANSON (1959b), a reduction in homology between two homologous chromosomes would decrease the effective map length so much that in a backcrossing program the selection for a desired character would be equivalent to the linkage-block breakup within a species having very short, approximately zero, map lengths. In *Gossypium* interspecific backcross transference, according to RHYNE (1958), desired recombinants were significantly fewer than the number expected from intraspecific hybrids. Linkage-block breakup during the backcross procedure approximated that of a species having very short map lengths; loci of the test-linkage group had the same linkage values in the tenth and greater backcross generations as in the first backcross generations from the interspecific hybrids. Linkage-block breakup was essentially unchanged by a large number of backcrosses into *G. hirsutum*, as if homology were reduced in the regions of the homologues carrying the test loci.

Linkage-block breakup, in backcross transference of desirable genes from related *Gossypium* species into *G. hirsutum*, could be difficult when the linkage block contained cryptic structural differences in chromosome homology (STEPHENS 1950). RHYNE (1960) reported that linkage blocks, or segments of linkage groups from diploid species, inserted into a *G. hirsutum* linkage group acted as structural units by increasing recombination in adjacent regions having pure *G. hirsutum* genes. This increase recombination probably represented a shift in chiasmata position by the inserted linkage block. A shift in chiasmata position away from a linkage block could make the linkage block persist. The breakup of persistent linkage blocks therefore confronts cotton breeders who wish to obtain desirable genes from diploid *Gossypium* species. The usage of X-radiation and

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mutagens on linkage blocks which have persisted through many backcross generations might not be the best way of enhancing linkage-block breakup. New structural changes as well as mutations could be induced haphazardly in the other linkage groups and thereby offset the gain obtained by repeated backcross transference.

The objective of this paper is to illustrate a possible genetic method for enhancing linkage-block breakup by increasing recombination within persistent linkage blocks following backcross transference in *G. hirsutum*.

MATERIALS AND METHODS

A number of linkage blocks from various *Gossypium* species, having essentially 0 map length, are acting as alleles to known *G. hirsutum* genes during repeated backcrossing to *G. hirsutum*. To show any increase of recombination and map length within these persistent blocks, certain allelic genes of *G. hirsutum* must be present in a heterozygous test genotype. Preferable alleles are those which are incompletely dominant so that any recombination phenotypes can be detected among the F_2 phenotypes. The anthocyanin alleles of the A genome linkage group 1, *R2-Yg2-Lc1*, and the D genome linkage group 3, *Cl1-R1-Yg1-dw*, can be manipulated as incompletely dominants. In addition, certain genes of diploid species act as alleles to various alleles at these loci. The genes employed herein follow:

Cl1—Noncluster or normal fruiting habit. The alleles *Cl^{rai}* and *Cl^{ari}*, respectively, from *G. aridum* (Rose & Standl.) Skovsted 2D₄ and *G. raimondii* Ulbr. 2D₅, were used in place of *Cl1* of *G. hirsutum*.

cl1—Cluster fruiting habit.

R1—Anthocyanin pigmentation in leaves and petal margins.

r1—Little anthocyanin in leaves and none in petal margins. The allele *r^{ari}* from 2D₄ causes pink to red, sometimes purple, petal margins and red anther cases, but *r^{thu}* from *G. thurberi* Tod., 2D₁ produces a phenotype very similar to that of *r1*.

Yg1—Not yellow-green leaves. The alleles *Yg^{ari}* and *Yg^{thu}*, respectively from 2D₄ and 2D₁, were used.

yg1—Yellow-green leaves when homozygous with *yg2*.

dw—White lint. The allele *dw^{rai}* from 2D₅ having "dirty-white," off-white to tan lint was used.

R2—Red anthocyanin petal spot, otherwise leaves as *r1* in phenotype. The alleles *R^{MG8}*, having anthocyanin in leaves and petal margins and tiny or no petal spot from *G. arboreum* L. 2A₂, and *R^{ogo}*, having a huge, intense red petal spot, but otherwise as *R2* from *G. anomalum* Wawr. & Peyr. 2B₁, were used.

r2—No petal spot. Leaves are the same as *r1* phenotype.

Yg2—Not yellow-green leaves. The allele *Yg^{ano}* from 2B₁ was used.

yg2—Yellow-green leaves when homozygous with *yg1*.

The following genotypes were assembled to demonstrate a change in map

length. An ordinary line represents *G. hirsutum* chromatin and the double line chromatin from a diploid species:

Genotype I $\frac{cl \ R1 \ yg1 \ dw}{Cl^{ari} \ r^{ari} \ Yg^{ari} \ dw}$ is a control genotype.

Genotype II $\frac{cl \ R1 \ yg1 \ dw^{rai}}{Cl^{ari} \ r^{ari} \ Yg^{ari} \ dw}$ had dw^{rai} inserted to increase recombination in the "ari" linkage block. The dw versus dw^{rai} recombination was not estimated for it served no special need in this paper.

Genotype III $\frac{Cl^{rai} \ R1 \ yg1 \ dw^{rai}}{cl \ r^{thu} \ Yg^{thu} \ dw}$ had Cl^{rai} and dw^{rai} inserted to increase recombination in region $r^{thu}-Yg^{thu}$.

Genotype IV $\frac{r2 \ yg2 \ Lc1}{R^{ogo} \ Yg^{ano} \ Lc1}$ is a control genotype, but only a few

plants carried $Lc1$, a brown-lint gene of *G. hirsutum*. The R^{ogo} causes a large, white petal spot on a yellow petal of *G. arboreum*, and interacts with $R2^{oos}$ spotless to make a red petal spot in F_1 hybrids of this species. R^{ogo} has been transferred to the $R1$ and the $R2$ linkage groups in *G. hirsutum*; it interacts with various $r1$ alleles to make a large red petal spot. When substituted for the $R1$ locus in homozygous condition, R^{ogo} interacts with $r2$ and makes a large red petal spot.

Genotype V $\frac{R2^{MGS} \ yg2 \ Lc1}{R^{ogo} \ Yg^{ano} \ Lc1}$ R^{MGS} was inserted to increase re-

combination within $R^{ogo}-Yg^{ano}$. The $R2^{MGS}$ can be placed in the $2B_1$ species at the R^{ogo} locus, for homology exists between A_2 and B_1 chromosomes.

Plants of each genotype were grown at Iguala, Mexico, in winter and the individual plants were self-pollinated. In summer in North Carolina the seed of each plant was planted and the populations were classified. The F_2 populations were used since the Iguala facilities were most efficient for inbreeding but were inadequate for large-scale backcrossing. The use of incompletely dominant genes in the close $R-yg$ linkages permitted a direct recognition of recombinations except for those of genotype IV. Calculated values reported in the tables, however, assume complete dominance of r to R ; values using the minimum discrepancy method correspond to those reported previously by the writer. In addition, the product-moment method was used to determine recombination values and standard errors.

EXPERIMENTAL RESULTS

The recombination values for genotype I, given in Table 1, indicate a small amount of recombination at region 1, $cl-R$, and essentially 0 at region 2, $R-yg$.

TABLE 2
*Recombination in hybrids of G. hirsutum having a persistent linkage block of
diploid G. anomalum*

Genotype	Recombination percent [†] <i>R2-yg</i>	Phenotypes in F ₂				Total plants in F ₂
		Petal spot present		Petal spot absent		
		<i>yg2yg2</i>	<i>Yg2 -</i>	<i>yg2yg2</i>	<i>Yg2 -</i>	
IV. <u><i>R20G0-Yg^{ano}-Lc1</i></u>						
<u><i>r2 yg2 lc1</i></u>	0.7a*	<i>R20G0 r2</i>	<i>R20G0-</i>	<i>r2 r2</i>	<i>r2 r2</i>	1059
	0.7 ± 0.3 pm	2	796	255	6(4 ?*)	
	0.6 md					
V. <u><i>R20G0-Yg^{ano}-lc1</i></u>						
<u><i>R2MGS-yg2 -Lc1</i></u>	0.6a*	<i>ROGOROGO</i>	<i>ROGOROGO</i>	<i>RMGSRMGS</i>	<i>RMGSRMGS</i>	
	0 pm	0	194	203	5(4 ?*)	
	0 md					
<i>R2 Yg2 Lc1</i>						
<u><i>r2 yg2 lc1</i></u>		<i>ROGORMGS</i>	<i>ROGOR^{MGS}</i>			<i>RHYNE</i> (1958, 1960) <i>intrahirsutum</i>
		0	438			
	20-25 md					

* The (?) recombinants could be contaminants since the best inbreeding method permits some pollen transmission by bees under field conditions. Testing did not answer the moot question.
† Actual percent of recombinations observed for total number of plants for incomplete dominance (a), by product-moment method (pm), and calculated by minimum discrepancy (md).

A second population of 780 plants for genotype A is not included in Table 1, for gene *vy*, virescent-yellow, was inadvertently brought in and might have confused the *Rr ygyg* classification. No *RRYg* plants appeared in the excluded 780, and the first and only recombinant between *R* and *Yg^{ari}* occurred in the 734 plants of Table 1, therefore, the 0.1% value, 1/734, is a maximum length for *r^{ari}-Yg^{ari}* when held heterozygous against *G. hirsutum* genes in a backcross population. The recombination was zero for this region of the genotype in previous backcrosses for similar population sizes (RHYNE, unpublished data).

A population of 196 plants was grown from genotype II. Populations of this size customarily are planted by many cotton breeders. Any increase in recombination for genotype II must show up in populations of this size so that breeders could detect recombinations. In Table 1 the recombination for genotype II has a 15 percent value at region 1 and a two percent value at region 2. The *dw^{rai}* thus increased recombination at region 1 to 15 percent, a value usually obtained within *G. hirsutum* varieties, and increased recombination from essentially zero to two percent at region 2. The two percent value is small compared with the range of 13–20 percent for *G. hirsutum* varieties, but experience shows it to be many times larger than the 0.1 percent of genotype I.

The genotype III recombination values in Table 1 are 32 percent for region 1 and 0.6 percent for region 2. Previous unpublished work, using the *r^{thu}-Yg^{thu}* segment held heterozygous against *G. hirsutum* genes in a genotype similar to genotype I, showed zero recombinants for 500 backcross plants. When all previous populations were combined, only one *R1-Yg^{thu}* and one *r^{thu}-yg1* recombinant were detected in more than 1300 plants. The control value for all these populations was a maximum of 0.2 percent. The value of test genotype III at *R-yg*, region 2, is significantly larger than this value. The segments having the *Cl^{rai}* and the *dw^{rai}* genes, respectively, were responsible for the significant increase of recombination at region 2 of genotype III. The 32 percent value for region 1 is unknown in pure *G. hirsutum*, although RHYNE (1960) reported a similar value when segment *Yg^{rai}-dw^{rai}* was inserted into the *cl-R-yg-dw* linkage group. The *r^{thu}-Yg^{thu}* linkage block can be considered to be persistent in comparison with the *R-yg G. hirsutum* linkage block.

In genotype IV of Table 2 the interest lies at *R^{ogo}-Yg^{ano}* which had no recombination of any of the preceding backcross generations. Recombinants are reported in Table 2, but in view of the zero value for all previous backcrosses in sizable populations and the elimination of several contaminants by marker genes obtained from other genetic stocks, the number of recombinants may be an overestimate. For example, from one inbred plant having *R^{ogo}-Yg^{ano}-Lc1/r-yg-lc1* an F₂ of 320 plants was grown. Three recombinants were observed—one was *r-Yg^{ano}-lc1*, an inescapable double recombinant, although no single had been detected before; and two were *R^{ogo}-yg-Lc1*, themselves possible double recombinants if the gene order for *R^{ogo}* were the same as *G. hirsutum*. A value of 0.7 percent was obtained for *R-yg* of genotype IV.

Genotype V had 0.6 percent recombination at *R-yg*. The *R^{ms}* linkage block probably did not alter recombination. The *R^{ogo}-Yg^{ano}* is a persistent linkage

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<u>r2 yg2 lc1</u>	0.7a*	R20G0 r2	R20G0-	r2 r2	r2 r2	1059
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V. <u>R20G0-Yg^{ano}-lc1</u>						
<u>R2MGS-yg2 -Lc1</u>	0.6a*	ROGOROGO	ROGOROGO	RMGSRMGS	RMGSRMGS	
	0 pm	0	194	203	5(4 ?*)	
	0 md					
R2 Yg2 Lc1						
<u>r2 yg2 lc1</u>		ROGORMGS	ROGORMGS			RHYNE (1958, 1960) <i>intrahirsutum</i>
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block within *G. hirsutum* linkage group 1. The $Yg^{ano}-Ic1$ map distance was not significantly different in genotype IV and genotype V and was within the range bracketing 32 percent as customarily found within *G. hirsutum*.

DISCUSSION

Backcrossing resulted in breakup of the $cl-R$ region of linkage group 3 for all allelic segments from *Gossypium* diploid species although at a much reduced rate as compared with pure *G. hirsutum*. The rate of breakup appeared to be unaltered by repeated backcrossing to *G. hirsutum* (RHYNE 1958). Backcrossing resulted, however, in a reduction of the length of the allelic segments of linkage group 1. Both the B_1 segment R^{ogo} and the A_2 segment $R2^{MGS}$ in linkage group 1 had been shorn of a gene that, in the early backcross generations, masked the expression of cluster ($cl1cl1$), a gene of linkage group 3 of the D_h genome. Nevertheless, 16 backcross generations were required before breakup within $R^{ogo}-Yg^{ano}$ was detected. This linkage block tied up 20–25 map units when held heterozygous against $R2-\gamma g2$ by backcrossing to *G. hirsutum*.

Linkage-block breakup was detected in genotypes II and III when the dw^{rai} of *G. raimondii* 2D₅ was inserted for dw of *G. hirsutum*. In each of these genotypes the recombination rate for the heretofore "completely-linked" $r-Yg$ allelic segments from the diploid species became a small positive value. In genotype III the inserted Cl^{rai} and dw^{rai} segments, as $Cl^{rai}-R-\gamma g-dw^{rai}$, increased recombination within the $cl-\gamma g$ region. However, the recombination values possibly might have been smaller than corresponding *G. hirsutum* values at $cl-R$ and $\gamma g-dw$, if the D_1 persistent block, $r^{thu}-Yg^{thu}$ were broken up. Instead, recombination increased significantly within $r^{thu}-Yg^{thu}$ and $cl-R$. The 32 percent value for $cl-R$ in genotype III was of the same magnitude as that reported by RHYNE (1960) when Yg^{rai} was substituted for γg of $cl-R-\gamma g-dw$. The $r^{thu}-Yg^{thu}$ is a persistent structural block of D_1 chromatin in *G. hirsutum* backcross heterozygotes.

The rate of breakup within $R^{ogo}-Yg^{ano}$ probably was similar in genotypes IV and V of Table 2. The effect of R^{MGS} from the A_2 genome in genotype V on $R^{ogo}-Yg^{ano}$ breakup probably was negligible. The lack of significant increase in breakup within $R^{ogo}-Yg^{ano}$ may have several explanations. The more likely ones can be: (1) the R^{MGS} segment from A_2 might have been too short to produce the desired increase, (2) the B_1 segment $R^{ogo}-Yg^{ano}$ could be a type (or degree) of structural segment, i.e., a different gene arrangement because of translocation or inversion, which precluded any increase in viable recombination products, and (3) together the A_2 and B_1 segments decreased recombination within $R2-\gamma g2$ and increased it distal to $R2$. This consequence could not be evaluated for no known gene has been assigned distal to $R2$. The unnamed gene "dwarf 1" however, shows weak linkage with $R2$ when certain alleles of $R2$ occur in the heterozygote, but when either R^{MGS} or $R^{ogo}-Yg^{ano}$ occurs in a similar heterozygote, the $R2$ and dwarf loci show independent inheritance (H. H. RAMEY and RHYNE, unpublished data).

In the present study several methods for inducing linkage-block breakup were employed. One method is related to the chiasmata shift phenomena reported by

RHYNE (1960), that diploid structural arrangements intercalated in a *G. hirsutum* linkage group increase recombination in pure *G. hirsutum* regions adjacent to the insertions. Therefore, presumably, in a heterozygote such as genotype II, $cl-R-yg-dw^{rai}/Cl^{ari}-r^{ari}-Yg^{ari}-dw$, chiasma position is altered and shifted into the vicinity of the *R* locus. Unnamed genes within the *Cl-yg* region of *G. hirsutum* in this heterozygote are combined with the Yg^{ari} genes of *G. aridum* 2D₄. When such unnamed genes are more desirable than many of their corresponding D₄ alleles, an increase in recombination within *cl-yg*, therefore, is a step in the right direction for eliminating unwanted D₄ genes. More backcrossing, or a repetition of the technique, may be required to obtain additional recombination within the small residual D₄ segment.

A second method can be useful for linkage-block breakup following interspecific hybridization and backcrossing to *G. hirsutum*. The variation depends on the greater homology of related diploid segments with each other than with the allelic *G. hirsutum* segment. For example, genotype V, $R^{ogo}-Yg^{ano}-lc1/R^{MGS}-yg2-lc1$, might be altered to contain the entire $R^{MGS}-Yg^{arb}2$ segment. Recombination might occur then within *R2-Yg2* because of greater homology in these longer segments than in the shorter segments of genotype V. Also, unnamed genes near Yg^{arb} may be less undesirable than the B₁ genes of Yg^{ano} and recombination would be in a favorable direction. Past experience has shown that *arboreum* genes can be recombined fairly readily with *hirsutum* genes and thus additional backcrossing with *G. hirsutum* could replace most of the *arboreum* genes. The variation would be most effective within *G. hirsutum* backcross background. Even so, a use of differential homologies could be made at the diploid level as $R^{ogo}-Yg^{ano}/R^{MGS}-Yg^{arb}$ in the B₁ × A₂ hybrid, at the amphidiploid level as the 2B₁D₁ × 2A₂D₁, hybrid, and at the hexaploid level as the 2A_hD_hB₁ × 2A_hD_hA₂, hybrid, and then crossing the offspring with *G. hirsutum*. One obvious limitation to the differential homology method is recombination combines structural blocks that may even be more resistant to breakup than the parental diploid blocks.

A third method is "forced" pairing in nonhomologous regions of hybrids and the use of large numbers in F₂ to detect new recombinations.

SUMMARY

Repeated backcrossing in *G. hirsutum* permits certain linkage blocks from diploid *Gossypium* species to persist and exhibit an essentially zero map length. Genetic methods for increasing recombination within these persistent linkage blocks of linkage groups 3 and 1 were illustrated. One method, using structural blocks of diploid *G. raimondii* 2D₅ inserted in linkage group 3, increased recombination in an adjacent *G. aridum* 2D₄ block from less than 0.1% to 2.0% and in a *G. thurberi* 2D₁ block from less than 0.2% to 0.6%. Concomitantly the D₅ block shifted chiasma position and increased recombination in the distal block cluster—red plant (*cl1-R1*) to 15.0 percent for the *G. aridum* block, and from less than 10.0 percent to 32.0 percent for the *G. thurberi* block. In pure *G. hirsutum* the recombination for *cl1-R1* is 15–17 percent. A second method, using differential homologies between allelic linkage blocks from *G. arboreum* 2A₂ and *G. anom-*

alum 2B₁ that had been inserted in linkage group 1, showed no significant increase in the persistent $R^{ogo}-Yg^{ano}$ block of 2B₁. The short *G. arboreum* segment $R2^{MGS}$ showed 0.6 percent recombination as compared to 0.7 percent for the *G. hirsutum* $r2-yg2$ segment when heterozygous against $R^{ogo}-Yg^{ano}$. A longer segment of *G. arboreum* possibly could alter recombination of persistent $R^{ogo}-Yg^{ano}$.

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