

# Breakup of Linkage Blocks in Cotton, *Gossypium hirsutum* L.<sup>1</sup>

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## ABSTRACT

We conducted this study to determine if the negative genetic associations frequently observed between yield and fiber strength in cotton (*Gossypium hirsutum* L.) could be reduced by random intermating. We selected for comparison two populations derived from a cross of 'Stoneville 7A' × 'Pee Dee 165'. The "original" population consisted of 96 families, each produced from a random F<sub>2</sub> plant. Ninety-six families comprised the "intermated" population. This intermated population was produced from two generations of random intermating after reaching F<sub>3</sub>.

Mean comparisons between the two populations showed small but significant differences for some traits, indicating that selection in the intermated population may have taken place or that linked epistasis was involved. Genotypic variances were approximately the same for both populations. The negative genetic correlation between lint yield and fiber strength was decreased by intermating. Genetic correlations between other traits were generally reduced by intermating. These results imply that some modifications of the conventional method of cotton breeding are necessary to improve yield and fiber strength. Modifications discussed are the use of random intermating, diallel selective mating, use of selection indexes, backcrossing, and bulk breeding.

**Additional index words:** Genetic correlation, Genetic variance, Breeding methods.

THE tri-species hybrid (*G. thurberi* Tod. × *G. arboreum* L. × *G. hirsutum* L.) produced by Beasley (3) introduced a new genetic source for higher fiber strength. Early breeding and selection with this material indicated a strong negative correlation between yield and strength. This could be attributable to either pleiotropic or linkage effects. Miller and Rawlings (7), working with populations derived from the cross of Empire 10 with TH 131-5, obtained results that implied that linkage was a contributing

cause for the negative genetic correlation between yield and strength. TH 131-5 was a strain that was closely related to the original triple hybrid. They recommended some intermating to break up linkage blocks for populations derived from exotic crosses. Since the production of the original triple hybrid, considerable breeding and selection for desirable yield and strength combinations have taken place. However, segregating populations involving high yield and high strength strains still show a negative genetic correlation.

The question then remains, "Will intermating within populations derived from improved triple hybrid material further reduce undesirable genetic associations?" The purpose of this study was to compare the effects of further intermating on reducing genetic correlations in such a population.

## MATERIALS AND METHODS

We used two populations derived from the cross of 'Stoneville 7A' and 'Pee Dee 165.' Stoneville 7A has excellent agronomic characteristics, high lint yield, and a wide area of adaptation. Pee Dee 165 yields less and has a smaller area of adaptation but has superior fiber properties. The performance of these two varieties in regional tests is given in ARS-34-113 (2). Pee Dee 165 descends from crosses of *G. hirsutum* L. and the tri-species hybrid (*G. thurberi* Tod. × *G. arboreum* L. × *G. hirsutum* L.). It differs from TH 131-5 used by Miller and Rawlings (7) in that it contains considerably more crossing to *G. hirsutum* L. in its pedigree and has had more selection for agronomic acceptability. The specific pedigree and characteristics of Pee Dee 165 has been reported (1). The populations compared in this study originated from F<sub>2</sub> seed produced by one F<sub>1</sub> plant.

The F<sub>2</sub> seed were produced at Stoneville in 1965. We selfed 150 F<sub>2</sub> plants in 1965-66 at Iguala, Mexico. The F<sub>3</sub> seed from each of the 150 F<sub>2</sub> plants were planted in progeny rows at Stoneville in 1966. Two F<sub>3</sub> plants in each progeny row were crossed with two other plants, each from a different F<sub>3</sub> progeny row. A total of 300 F<sub>3</sub> plants were used to produce 150 crosses for this first intercross generation. In 1967 the bulk was planted two seeds per hill, 32 cm apart. Each hill was thinned to one plant. The second generation of intercrossing produced 250 crosses from 500 plants. A bulk was formed from these crosses by taking one seed from each cross. Ninety-six individual plants were grown from this bulk in Iguala, Mexico. Selfed seed of these 96 individually harvested plants constituted the "intermated" population. All crosses were made at random within the restraints given.

Remnant seed of the 1965 F<sub>2</sub> seed were kept in cold storage till planting at Stoneville in 1967. Ninety-six F<sub>2</sub> plants grown

<sup>1</sup>Contribution from the Delta Branch of the Mississippi Agricultural and Forestry Experiment Station, in cooperation with Plant Science Research Division, Agricultural Research Service, U. S. Department of Agriculture, Stoneville, Miss. Published as Journal Paper 2111 of the Mississippi Agricultural and Forestry Experiment Station. Received March 5, 1971.

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from this seed were selfed and the  $F_3$  progenies were tested as the original population. Therefore, two populations were derived: the intercrossed, involving two intercrosses after reaching  $F_3$ , and the original, involving  $F_3$  progenies. Their mean expectations would be the same, assuming that there was only additive and dominance gene action, no type of linked epistasis, and no selection.

The two populations were divided into six sets, each set consisting of 16 random original progenies and 16 random intermated progenies. Two entries each of the two parental varieties, Stoneville 7A and Pee Dee 165, were included in each set. The material was grown at two locations at Stoneville in 1968, one with a sandy soil, and the other with a clay soil. A randomized complete block design with two replications per location was used for each set. Plots were single 13.3-m rows, 1 m apart. Seeding rate was one seed per 10-cm row planted in hills 32 cm apart.

One 100-boll sample was taken from each plot. Lint percentage was determined from saw-ginned samples. The lint percentage of each plot was multiplied by the plot's seed cotton yield to obtain the lint yield per plot. The average weight in grams per boll of the samples was used to estimate boll size. The weight of 100 seeds in grams from each sample was used to obtain seed index estimates.

Fiber length was measured as 50% and 2.5% span length on a Digital Fibrograph. Strength ( $T_1$ ) expressed as grams per tex and elongation ( $E_1$ ) were measured with the  $\frac{1}{8}$ -inch gauge Stelometer. Fiber fineness was expressed in micronaire units. Fiber determinations were made by the US Cotton Fiber Laboratory of the Plant Science Research Division, Agricultural Research Service, USDA, at Knoxville, Tenn.

Estimates of the components of variance and covariance among progenies were obtained in separate analyses for the two populations. The pertinent analysis of variance and covariance and the mean square or mean product expectations are presented in Table 1.

The components were used to estimate the genotypic variances and correlations, which is the additive genetic correlation if all variance is additive.

$$\text{Genotypic } r_{ij} = \sigma_{p_{ij}} / (\sigma_{p_{ii}} + \sigma_{j_{jj}})^{1/2}$$

Standard errors for genetic variances and for genetic correlation coefficients were computed by the methods given by Mode and Robinson (8).

## RESULTS AND DISCUSSION

### Means

Performance of the parental varieties is given in Table 2. Significant differences for all traits except micronaire were detected. Yield and fiber property differences were not as large as those reported by Miller and Rawlings (7). This probably accounts for some of the differences encountered between the two studies.

Data for the two populations are given in Table 3. We found small but significant differences for five of the nine traits. The mean performance of the original population was higher for yield, lint percent and boll size. The higher lint percent accounts for most of the higher lint yield. The intermated population had a higher seed index and fiber strength. With the exception of boll size, differences correspond to the intermated population shifting toward Pee Dee 165. Differences between populations might arise

from three sources. Epistasis involving linkage is one possibility, but in general, epistasis has not been found to be of great importance in cotton. The seed lots for the two populations were produced under different environments. The original population was produced at Stoneville and the intermated, at Iguala, Mexico. Both seed groups were of excellent quality. Stand counts and vigor ratings shortly after seedling emergence indicated no difference for the two populations. The third factor that could result in the small, but significant differences is selection. Miller and Rawlings (7) also encountered differences between their two populations, except that the intermated was superior in yield. They postulated that selection may have inadvertently entered into their experiment. This possibility cannot be ruled out in these tests either. We can only speculate that selection also has entered into these studies.

### Variances

An  $F_2$  population is not in linkage equilibrium. Hanson (5) has shown that  $F_2$  genetic variances may vary considerably from those expected for genetic equilibrium. Crosses with a predominant coupling-phase linkage would be expected to have reduced genetic variances upon intermating. Genetic variances would be expected to increase for those crosses with a predominant repulsion-phase linkage. While significant changes in genetic variances indicate the presence of linkage, nonsignificance does not necessarily indicate nonlinkage. In general, the genetic variances given in Table 4 show little differences between the two populations. Slight reductions for lint yield and seed index were observed. In the study by Miller and Rawlings (7) the trend was for a reduction in genetic variances. Their parental lines possessed greater differences and their high-strength parent was closely related to the trispecies hybrid. Under these conditions one might expect predominant coupling-phase linkages and greater reductions in genetic variances. As more breeding and selection was practiced, the predominant coupling phase probably would be reduced. Such might be the case for the cross of Stoneville 7A and Pee Dee 165.

Table 2. Performance of parental varieties.

Trait	Stoneville 7A	PD 165	PD/7A:100
Lint yield, kg/ha	1,157**	978	84.5
Lint, %	39.0**	37.7	96.8
Boll size, g	5.21	5.84**	112.1
Seed index	10.2	12.2**	119.6
50% span length	.511	.536**	104.9
2.5% span length	1.107	1.121**	101.3
Strength, $T_1$	18.5	22.2**	120.0
Elongation, $E_1$	6.1**	5.3	86.9
Fineness, Mic.	4.81	4.73	98.3

\*\* Significantly higher than the other parent at the .01 level.

Table 1. Pertinent portion of analysis of variance and covariance between traits  $i$  and  $j$ .

Source	df	Mean product expectation*
Progenies in sets	$s(p-1) = 90$	$\sigma_{ij} + r \sigma_{plij} + r l \sigma_{ptj}$
Progenies in sets $\times$ loc.	$s(p-1)(l-1) = 90$	$\sigma_{ij} + r \sigma_{plij}$
Error	$sl(p-1)(r-1) = 180$	$\sigma_{ij}$

\*  $\sigma_{ij}$  = pooled error.  $\sigma_{plij}$  interaction covariance (variance) of progenies  $\times$  locations.

$\sigma_{ptj}$  = genotype covariance (variance) for progenies.  $r$  = number of replications.  $l$  = number of locations.  $p$  = number of progenies.  $s$  = number of sets.

Table 3. Comparison of original and intermated population means.

Trait	Original	Intermated
Lint yield, kg/ha	1,140**	1,076
Lint, %	38.3**	37.7
Boll size, gm	5.51**	5.39
Seed index	11.5	11.6**
50% span length	.532	.537
2.5% span length	1.129	1.129
Strength $T_1$	20.5	20.9**
Elongation, $E_1$	5.8	5.8
Fineness, Mic.	4.60	4.58

\*\* Significantly higher at the .01 level than the other population mean.

### Genotypic Correlations

Genotypic correlations for all 36 combinations of nine traits for both populations are given in Table 5. Genotypic correlations involving lint yield are of primary importance. The component of yield that most frequently has been highly correlated with yield has been lint percent. A correlation of .70 was obtained in both populations for these two traits in this study. Correlations of boll size and seed index with yield were reduced from  $-.43$  to  $-.13$  and from  $-.45$  to  $-.30$ , respectively, after intermating.

The main objective of this cross, to combine yield traits of Stoneville 7A with the higher strength ( $T_1$ ) of Pee Dee 165, was realized to some extent. This unfavorable correlation was reduced from  $r = -.54$  to  $r = -.38$  by the intermating procedure. A reduction from  $r = -.69$  to  $r = -.35$  was obtained by Miller and Rawlings (7). The genetic correlations obtained after intermating compare closely for the two studies.

The change in genotypic correlations for lint yield and the two measurements of length were not consistent. The correlation of lint yield and 50% span length was reduced from  $r = -.43$  to  $r = -.28$ . For yield and 2.5% span length an increase from  $r = -.47$  to  $r = -.67$  was obtained. An increase in a genetic correlation coefficient can be obtained if linkages were in a predominant repulsion phase. Similar results were obtained by Miller and Rawlings (7). It should be pointed out that the 50% and 2.5% span length are not necessarily measuring the same length parameters. The genetic correlation between 50% and 2.5% span length was lower in the intermated population. For most traits that had high genotypic correlations in the original population, correlations were found to be lower in the intermated population. For example, of the 10 highest correlations in the original population, eight showed reductions; one, an increase; and one, no change in the intermated population. This study indicates that in this population, linkage is still a contributing factor to the difficulties in improving both yield and strength of cotton.

### Breeding and Selection Implications

Cotton breeding methods probably are not as well defined as those of other predominantly self-pollinated crops. In general, the pedigree method has been used most frequently. Selection usually starts with  $F_2$  plants followed by progeny rows and more plant selections in subsequent generations. Cotton breeders selecting for higher yield and fiber quality have not been satisfied with the results of this approach. A

high selection intensity in the early generations usually has resulted in very few desirable recombinants. Some modifications of the conventional system seem necessary.

Random intermating has been suggested by Hanson (4) and by Miller and Rawlings (7). This method would produce the most recombinants when linkage is involved, but its disadvantage is that no genetic advance is made during the several generations of intermating. The availability to breeders of large gene pools that have been produced by random mating procedures would greatly reduce this limitation. Jensen (6) has proposed a diallel selective mating system to overcome some of the difficulties of the conventional breeding system. Multiple parents are used to form a large gene pool, which, through selective matings of individuals, is advanced through successive generations. This procedure would break up linkage blocks by intermating and provide a large gene pool but at the same time allow genetic advance. A third procedure that might be adopted in the  $F_2$  or  $F_3$  generation is the use of selection index procedures. The index value would be determined by the weighted yield and strength values. Progenies selected by this procedure, while having similar economic values, could be greatly different in phenotype and genotype. These selections could be crossed in  $F_4$  to start the next breeding cycle. This selection procedure could be incorporated into Jensen's (6) breeding method.

A fourth alternative is backcrossing. Fiber strength has high heritability, low genotype-by-environment interactions, and can be determined at a relatively low cost. The higher-yielding parent would be used as the recurrent parent and the high-strength parent, as the donor parent. The objective would not be to produce a near-isogenic line but to establish a breed-

Table 5. Genotypic correlations ("r" values) between pairs of traits in the original and intermated populations.

Traits correlated	Genotypic r			
	Original		Intermated	
	r	SE	r	SE
Lint yield vs lint %	.70 (.14)		.70 (.16)	
vs boll size	-.43 (.22)		-.13 (.23)	
vs seed index	-.45 (.15)		-.30 (.19)	
vs 50% SL	-.43 (.23)		-.28 (.23)	
vs 2.5% SL	-.47 (.18)		-.67 (.19)	
vs $T_1$	-.54 (.17)		-.38 (.19)	
vs $E_1$	.03 (.20)		-.15 (.20)	
vs micronaire	.42 (.19)		.65 (.18)	
Lint, %				
vs boll size	-.18 (.12)		-.07 (.13)	
vs seed index	-.69 (.07)		-.53 (.14)	
vs 50% SL	-.39 (.13)		-.32 (.12)	
vs 2.5% SL	-.55 (.09)		-.47 (.09)	
vs $T_1$	-.33 (.10)		-.36 (.08)	
vs $E_1$	.00 (.04)		.01 (.12)	
vs micronaire	.37 (.14)		.48 (.13)	
Boll size				
vs seed index	.71 (.19)		.66 (.10)	
vs 50% SL	.12 (.18)		-.06 (.16)	
vs 2.5% SL	.15 (.14)		.13 (.12)	
vs $T_1$	-.01 (.14)		.20 (.14)	
vs $E_1$	-.26 (.15)		.09 (.14)	
vs micronaire	.02 (.14)		-.04 (.14)	
Seed index				
vs 50% SL	.69 (.11)		.55 (.11)	
vs 2.5% SL	.60 (.09)		.49 (.10)	
vs $T_1$	.51 (.09)		.55 (.09)	
vs $E_1$	-.03 (.14)		.03 (.13)	
vs micronaire	-.16 (.13)		-.30 (.12)	
50% SL				
vs 2.5% SL	.82 (.08)		.65 (.11)	
vs $T_1$	.77 (.04)		.72 (.12)	
vs $E_1$	.02 (.07)		.23 (.14)	
vs micronaire	.09 (.15)		-.05 (.05)	
2.5% SL				
vs $T_1$	.41 (.10)		.45 (.10)	
vs $E_1$	.07 (.04)		.14 (.12)	
vs micronaire	-.42 (.14)		-.52 (.07)	
$T_1$				
vs $E_1$	.03 (.14)		.01 (.12)	
vs micronaire	-.21 (.13)		-.26 (.12)	
$E_1$				
vs micronaire	-.40 (.10)		-.34 (.11)	

Table 4. Genotypic variances of original and intermated populations.

Trait	Original		Intermated	
	$\sigma^2$	SE	$\sigma^2$	SE
Line yield, kg/ha	6,639	(2,522)	4,311	(2,016)
Lint, %	2,278	(.3596)	1,906	(.3009)
Boll size, gm	.0834	(.0202)	.0947	(.0214)
Seed index	.5505	(.0945)	.3724	(.0654)
50% SL	.00016	(.000045)	.00024	(.000054)
2.5% SL	.00076	(.000138)	.00090	(.000152)
$T_1$	.9432	(.1653)	.9631	(.1664)
$E_1$	.0752	(.0163)	.0947	(.0170)
Micronaire	.0697	(.0119)	.0655	(.0115)

ing population from which to select more desirable recombinants of yield and strength.

Under the conventional system of breeding, strong early selection for either yield or strength separately tends to produce very few desirable recombinants. Larger populations and less early selection therefore would be desirable. One practical solution would be to use a modified bulk in the early generations. A large  $F_2$  population would be grown. One locale would be harvested from each plant. The seed from all plants would be bulked for the next generation. Repeat the procedure for the  $F_3$  generation. Selection would be delayed until the  $F_4$  generation. Plant selections based on type and lint percent would then be initiated. This procedure would allow handling large numbers of plants at low cost and result in a greater frequency of recombinants. In addition, in the areas such as the Mississippi Delta, where cotton is essentially self fertilizing, the additive and additive-by-additive genetic variances would be 75 and 206% greater, respectively, than in the  $F_2$  population. The modified bulk procedure could be carried on with lit-

tle effort and expense at the same time as some of the other proposed modifications were being used.

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