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# Phenotypic Stability of Cotton[1]

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## ABSTRACT

**We studied the effects of level of ploidy and of heterozygosity on the phenotypic stability of certain inbred lines of cotton (*Gossypium* sp.) and their $F_1$ hybrids. The experimental material consisted of four inbred lines from each of three cultivated species of cotton. These species were an Old World diploid (*G. arboreum* L.), and two New World allotetraploids (*G. hirsutum* L. and *G. barbadense* L.). Eight phenotypic characters or traits were measured in five environments. In each environment the material was grown in two randomized blocks, divided into split-plots. Inbreds and their $F_1$ hybrids were used to minimize genetic variance, and estimated environmental variances were analyzed.**

**The level of ploidy did not significantly affect the phenotypic stability of the eight characters. The diploid *G. arboreum* was as well buffered against environmental variation as the two allotetraploids, *G. hirsutum* and *G. barbadense*. Thus, the intergenomic heterozygosity associated with amphidiploidy conferred no advantage to plants by enhancing their ability to adjust to fluctuations in the environment. Significant differences in phenotypic stability usually were not found in comparisons of inbred lines ($F_0$) and hybrid progenies ($F_1$). The expression of phenotypic stability appeared to be random with respect to five specific environments in which the plants were grown. Apparently the cultivated species of cotton we studied do not possess homeostatic systems associated with either homozygosity or heterozygosity.**


***Additional index words:*** ***Gossypium,*** **Developmental homeostasis, Plasticity, Ploidy level.**

PHENOTYPIC stability is defined in this paper as the ability of individual cotton plants to buffer environmental fluctuations so as to maintain uniform patterns of development. Phenotypic stability was measured as the variation between individuals with like genotypes (i.e., highly inbred lines or $F_1$ hybrids).

Geneticists have studied phenotypic stability in the cultivated allotetraploid species of cotton, *G. hirsutum* (Kohel and White, 1963; Kohel, 1969). Their results suggested that individuals from inbred lines and $F_1$ hybrid populations do not differ significantly in their capacity to minimize environmental fluctuations and thereby maintain stable phenotypes. Kohel (1969) proposed that the absence of a difference in phenotypic stability between homozygotes and heterozygotes could be related to the form of heterozygosity found between homeologous genomes in the amphidiploid *G. hirsutum*.

The purposes of this study were (i) to determine the effects of ploidy on phenotypic stability and (ii) to evaluate further the stability associated with parental lines and $F_1$ hybrids under several discrete environments.

## LITERATURE REVIEW

Donald (1948) suggested that a departure from the natural breeding system of a species would reduce the ability of the individual to buffer environmental changes. This failure in environmental buffering would be reflected by an increase in the amount of nongenetic (environmental) variation occurring between individuals.

Lerner (1954) employed the term developmental homeostasis to refer to the environmental buffering associated with adaptive characters. He alleged that conclusions as to the preferred state of heterozygosity or homozygosity could be inferred only if traits with

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known adaptive significance were used. Thus, the primary difference between the definition of phenotypic stability and developmental homeostasis involves whether or not characters measured have adaptive significance. We use the term phenotypic stability because of the difficulty in determining the adaptive significance of many of the characters studied in cotton.

Lerner consolidated and organized data from outbreeding organisms, and concluded that hererozygosity minimized the effects of environmental changes and therefore was the preferred state in outbreeding species. The greater phenotypic stability of the heterozygote has been shown in outbreeding organisms with the demonstration that a heterozygote has a smaller environmental variance than a homozygote (Adams and Shank, 1959; Chai, 1957; Dawson, 1968; Dodzhansky and Levene, 1955; Mather, 1953).

Lerner (1954) recognized the fact that very little information was available from inbreeding organisms. But he suggested that, in inbreeding species, the homozygote should be more stable than the heterozygote, because the natural breeding system would tend to maximize homozygosity. Experiments conducted to test Lerner's hypothesis have shown that greater phenotypic stability is not associated exclusively with the homozygous condition in inbreeding species; instead, such studies have shown that the environmental variance, associated with homozygous individuals, is not consistently smaller or larger than the variance associated with heterozygous individuals (Jinks and Mather, 1955; Lewis, 1954; Paxman, 1956; Williams, 1959; 1960).

## MATERIALS AND METHODS

Four of the 32 currently recognized species of *Gossypium* are represented by cultivated agricultural varieties. Two of these are diploids, *G. arboreum* and *G. herbacum*, (n=13; genome designation: *A*), and two, *G. hirsutum* and *G. barbadense*, are allotetraploids (n=26; genome designation: *AD*). Although representatives of all of the species of cotton would have been desirable in this study, those of *G. herbacum* and of the 28 wild species were eliminated from consideration because previous experience at this station had shown them to be so ill-adapted as to make their culture impractical, even under experimental conditions.

We selected four lines of *G. arboreum* as representatives of three cultivated biotypes of this species: 'Nanking' var. *typicum*; 'CB 787' and 'Bengal' var. *neglectum*; 'Garo Hill' var. *cernuum* (Hutchinson and Ghose, 1937). These lines have been maintained by pedigree self-pollination.

The four varieties of *G. hirsutum* selected for study were 'Deltapine Smooth Leaf,' 'Stoneville 7A,' 'Carolina Queen,' and 'Rex Smooth Leaf.' Deltapine Smooth Leaf and Stoneville 7A were developed primarily for the Mississippi Delta, but the wide adaptation of these varieties has allowed them to be grown in most of the rain-fed areas of the US Cotton Belt. Carolina Queen was developed primarily for the eastern portion of the Cotton Belt, and the Rex Smooth Leaf variety was developed for northern upland areas of Arkansas. All of these varieties are commercially acceptable Upland cotton types and are highly selected, relatively inbred lines. Breeder seeds of each variety were obtained from the respective breeders.

American-Pima varieties were chosen as representatives of *G. barbadense*. We used four lines of American-Pima: '3-79,' 'Pima S-2,' 'Pima S-3,' and 'Pima S-4.' The stock known as 3-79 is used in genetic studies on *G. barbadense* at both the Arizona and Texas Agricultural Experiment stations. Line 3-79, derived by doubling a haploid from the strain 3-79, has been maintained by controlled self-pollination for many generations. Breeder seeds of the commercially grown American-Pima varieties Pima S-2, S-3, and S-4 were obtained from the Cotton Research Center, Arizona Agricultural Research Station, Phoenix, Ariz.

All possible crosses were made among lines within each species, and seeds from reciprocal crosses were bulked. Thus, there were four parental lines and six $F_1$ hybrids per species for a total of 30 genetically nonsegregating entries per replication. Each entry was represented by five plants per plot. The entries were arranged in split plots with two replications. Species represented whole plots, and entries (parents and hybrids) within species represented split plots. The within-plot variance of each entry was computed to estimate the environmental variance as a measure of phenotypic stability. These environmental variances were converted to logarithms, and the transformed data were analyzed: (i) among species, to determine the effects of ploidy level on phenotypic stability; and (ii) within species, to indicate whether the inbred lines or the $F_1$ hybrids showed greater phenotypic stability.

The 30 entries were grown in five different environments. Seeds from each entry were germinated in 6-ounce paper cups in the greenhouse and transplanted 2 weeks after the initial planting. The experimental material was grown in the following five discrete environments:

(1) and (2), *1968 and 1969 field plantings*. The 1968 growing season was particularly wet with above-average rainfall during the entire season. Rainfall was well below normal during the 1969 growing season, and plants suffered periods of moisture stress between irrigations. In each of these environments seedlings were transplanted into 1 × 10-m rows, spaced .46 m apart (20 plants per row), and subjected to routine cultural practices. Miscellaneous plants were set out at the end of each row to minimize border effects.

(3), (4), and (5), *1969 Spring and Fall greenhouse plantings*. Environments 3 and 4 were early spring greenhouse plantings. A minimum temperature of 20 C was maintained in the greenhouse for environment 3 and a minimum temperature of 27 C was maintained in environment 4. Supplemental heat was not required during the early growing period of environment 5, but later the minimum temperature was maintained at 25 C. Seedlings were transplanted into 30.5-cm (12-inch) equally spaced plastic pots (20 pots on a .4 × .6-m steel bench). They were filled with equal parts of peat moss, pearlite, and soil in all three greenhouse plantings. Plants were watered often enough to minimize moisture stress.

We measured the following eight characters on individual plants:

(1) *Pistil length* — average length, in millimeters, of two pistils from the tip of the stigma to the base of the style.

(2) *Ovule number per locule* — ovule attachments in one locule from each of three bolls were counted and averaged.

(3) *Boll size* — weight in grams of seed cotton per boll.

(4) *Leaf dimension ratio* — leaf width/leaf length from three leaves were measured and averaged.

(5) *Yield* — grams of seed cotton harvested.

(6) *Number of bolls* — per plant.

(7) *Days to first flower* — determined by daily observations.

(8) *Mean maturity data (MMD)* — a measure of the relative maturity of the plant (Christidis and Harrison, 1955).

## RESULTS AND DISCUSSION

Among-species analyses of environmental variances revealed significant differences between species in pistil length, boll size, boll number, days to first flower, and mean maturity data; however, these differences were not related to the level of ploidy (Table 1). The diploid *G. arboreum* showed as much phenotypic stability as the two allotetraploid species. *G. arboreum* was more stable in pistil length and boll size, less

**Table 1. Mean environmental variance of each character in each species.**

| Characters | Species | | |
|---|---|---|---|
| | G. arboreum | G. hirsutum | G. barbadense |
| Pistil length | 11.3 b* | 12.9 b | 31.0 a |
| Ovule number | 1.5 a | 1.2 a | 1.9 a |
| Boll size | 0.5 c | 2.2 a | 0.8 b |
| Leaf dimension ratio | 142.1 a | 115.3 a | 145.2 a |
| Yield | 537.1 a | 813.2 a | 525.1 a |
| Boll number | 100.1 a | 43.6 c | 69.2 b |
| Days to 1st flower | 89.2 b | 63.1 c | 128.3 a |
| MMD | 66.1 a | 51.3 a | 22.9 b |

* Means within a row having a letter in common are not significantly different at the .05 level according to Duncan's Multiple Range Test.

**Table 2. Analysis of environmental variances within each species.**

| Source | df | Mean squares | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hir. | Arb. | Barb. | Hir. | Arb. | Barb. | Hir. | Arb. | Barb. | Hir. | Arb. | Barb. |
| | | Pistil length | | | Ovule number | | | Boll size | | | Leaf dim. ratio | | |
| Environment | 4 | .32 | .54 | .48 | .53* | .15 | .10 | .13 | .49 | .51 | .16 | .18 | .08 |
| Reps/env. | 5 | .12 | .06 | .27 | .06 | .11 | .11 | .42 | .11 | .16 | .24 | .09 | .20 |
| Entries | 9 | .24 | .17 | .16 | .06 | .27** | .07 | .30 | 1.28** | .26 | .20 | .09 | .24 |
| $F_0$ vs $F_1$ | 1 | .18 | .14 | .94** | .06 | .02 | .07 | .09 | .11 | .13 | .11 | .48* | .23 |
| Env. × entries | 36 | .16* | .13 | .08 | .09 | .08 | .09 | .19 | .20 | .14 | .15 | .16 | .12 |
| Reps/env. × entries | 45 | .09 | .10 | .11 | .08 | .09 | .14 | .18 | .13 | .18 | .11 | .10 | .19 |
| | | Yield | | | Boll number | | | Days to flower | | | MMD | | |
| Environments | 4 | 6.77** | 7.33** | 5.52** | 4.23** | 6.68** | 5.30** | 3.40** | 3.23** | 3.61** | 1.08 | 1.35 | 3.82** |
| Reps/ env. | 5 | .31 | .25 | .14 | .19 | .30 | .22 | .32 | .19 | .20 | .24 | .38* | .33 |
| Entires | 9 | .23 | .45* | .14 | .32* | .93** | .17 | .30 | .31 | .24 | .21 | .41 | .21 |
| $F_0$ vs $F_1$ | 1 | .17 | 1.51** | .19 | .11 | .12 | .13 | .18 | .28 | .78* | .47 | .36 | .13 |
| Env. × entries | 36 | .18 | .18 | .19 | .15 | .21 | .26 | .20 | .36* | .23 | .18 | .27* | .24 |
| Reps/env. × entries | 45 | .18 | .19 | .15 | .12 | .17 | .16 | .17 | .19 | .19 | .19 | .15 | .26 |

*, ** Significant at .05 and .01 levels, respectively.

stable in boll number and mean maturity date, and was intermediate between the allotetraploid species in the other characters.

Analyses of the environmental variances within species showed that the inbred lines (homozygotes) and $F_1$ hybrids (heterozygotes) did not differ in their phenotypic stability. Four of the $F_0$ vs $F_1$ comparisons were significant (Table 2), but these comparisons did not constitute a pattern from which we could draw meaningful inferences. We arranged the within-species variances so that we could make individual environmental comparisons, and thus, determine the most stable state within, whether $F_0$ or $F_1$. The results showed that, regardless of the character or species, in some environments the homozygous individuals ($F_0$) were more stable; in others, heterozygous individuals ($F_1$) were more stable. These data show a random pattern: we observed 60 cases of $F_0$ stability, 57 of $F_1$ stability, and 3 in which the $F_0$ and $F_1$ did not differ in phenotypic stability.

Kohel (1969) suggested that the internal heterozygosity between homeologous genomes of an allotetraploid may increase the time required for a truly homozygous condition to develop. This internal heterozygosity was offered as a possible explanation for the lack of a difference in phenotypic stability between inbred lines and hybrid progenies in *G. hirsutum*. Our data show that when this comparison was tested experimentally (i.e., when a diploid species is compared with an allotetraploid species) there was still no difference in phenotypic stability.

Our tests showed that not one of the three species was more phenotypically stable in the heterozygous condition than the others. On the contrary, the inbred lines appeared to be developmentally as stable as the hybrids. Furthermore, there was no pattern of stability (other than random) that could be associated with any of the varied environments in which the plants were grown.

Our results suggest that the three species of *Gossypium* we studied have not developed a pronounced (or even measurable) homeostatic mechanism related to their natural breeding system. In view of the fact that these species have an apparatus for encouraging cross-pollination (showy flowers, floral nectaries, spiny pollen), and also an effective method of self-pollination (proximity of the staminal column to the stigma, complete self-fertility) the lack of a homeostatic mechanism should not be unexpected. These species of *Gossypium* seem to have evolved a breeding system that favors self-fertilization for fitness to specific environments. But at the same time, the system does not exclude some cross-fertilization that will provide genetic variants that can survive in new environments. Under these conditions, there is probably no strong natural selection pressure to fix homeostasis in either the homozygous or heterozygous state.

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