Phenotypic Stability of Homozygous Parents and Their F_1 Hybrids in Upland Cotton, *Gossypium hirsutum* L.[1]

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ABSTRACT

Experimental material consisted of nine parental lines of cotton, *Gossypium hirsutum* L., and their F_1 progenies in a complete diallel crossing system. Eight parents were haploid derived lines and one was a highly inbred line. The individual entries were grown in five-plant plots, replicated five times, and grown in 2 years. Plant height, earliness, seed index, lint percentage, and yield were measured on an individual plant basis. The logarithmic transformation of the within-plot variance was computed for each character to measure phenotypic stability.

There was no clear-cut distinction between the variability of the parents and hybrids, even though the parents were less variable for three of the five characters measured. The effect of the evolution of self-fertilization on the homeostatic mechanism is discussed. It is suggested that self-fertilized organisms may be in a transitional state, and they may not have developed a distinctive homeostatic mechanism such as that found in cross-fertilizing organisms.

Additional index words: Homeostasis, Self-fertility.

EARLY in this century Shull (1908) and East (1908), working with corn (*Zea mays*), demonstrated the superiority of the F_1 hybrid over parental lines and other populations with varying degrees of heterozygosity. The terms "hybrid vigor" and "heterosis" were coined to represent this phenomenon. Heterosis was recognized in animals as well as in plants, and attempts to use it in the improvement of performance of both types of organisms led to new breeding concepts. In the subsequent search for the underlying genetic mechanisms that govern the expression of heterosis, researchers began to recognize that heterotic expressions were being found in plants, and certainly in animals, in which outbreeding was the natural breeding system. After recognition that species which characteristically display heterosis are often outbreeders, which normally maintain high degrees of heterozygosity in natural populations, further investigation disclosed that the F_1 hybrids possessed unique stability by being buffered against environmental variation. The stability of heterozygotes in outbreeding organisms has been demonstrated through experimentation; but as in the study of heterosis, the underlying mechanisms have not been fully explained.

Donald (1948) suggested that departures from the natural breeding system reduce the stability of the developmental process. I conducted this experiment with a *usually self-fertilized* species, *Gossypium hirsutum* L., and data are presented on the phenotypic stability of homozygous parents and their F_1 hybrids.

LITERATURE REVIEW

Lerner (1954) reviewed the literature relating to experiments which contained information on the stability of heterozygotes.

He consolidated and organized the data, both pro and con, and put together a comprehensive exposition on the phenomenon, including an explanatory model, and proposed a standard set of nomenclature. This phenomenon had been referred to as balance, buffering, and homeostasis of heterozygotes. Lerner adapted the term homeostasis as relating to the general phenomenon, and further defined developmental and genetic homeostasis as referring to individuals and populations, respectively.

Lerner (1954) noted that outbreeding populations, by the very nature of their breeding system, tended to maximize the level of heterozygosity, and that through natural selection, maximized heterozygosity became most advantageous. The homeostatic advantage of the heterozygote is demonstrated by its ability to buffer against environmental changes. This buffering capacity may be demonstrated experimentally by showing that the F_1 has a lower environmental variance than progenies with lower levels of heterozygosity.

Various experiments have been designed to measure and evaluate the role of homeostasis in animals. *Drosophila* spp. (Wigan, 1944; Robertson and Reeve, 1952; Dobzhansky and Wallace, 1953; and Mather, 1953) and mice (Yoon, 1955; McLaren and Michie, 1956; and Harrison, 1962) have been used as experimental organisms; the results demonstrated homeostasis in F_1 populations.

Lerner (1954) recognized the lack of definitive information from the plant kingdom, but he suggested, from the information available, that the concept of homeostasis and its relation to the natural breeding system remained valid and that outbreeders were characterized by homeostatic F_1's and inbreeders by homeostatic inbred lines.

Mather (1950) reported a study of heterostyly, which determined reproductive fitness, in the outbreeder, *Primula sinesis*. He found that environmental variances of the F_1 generation were less than those of the parental lines. Adams and Shank (1959) conducted an experiment designed specifically to detect homeostasis in maize. They used several maize lines that varied in origin and in previous mating system to produce varying degrees of homozygosity and heterozygosity. Their results demonstrated that as the degree of heterozygosity of the hybrid increased, the environmental variability of the F_1 decreased. These results fit Lerner's homeostatic model.

Jinks and Mather (1955) and Paxman (1956) conducted experiments utilizing *Nicotiana rustica* and Williams (1960) utilizing *Lycopersicon esculentum* to study the homeostatic properties of inbreeding species. In these experiments, the variances of the F_1's did not differ significantly from the variances of the parents, and occasionally they exceeded them. Data presented by Hagberg (1952) for *Galeopsis*, an inbreeder, showed no apparent differences in variability between parental lines and F_1 progenies in most cases. Griffing and Langridge (1963) measured the variability of fresh weight in the obligate self-fertilizing species *Arabidopsis thaliana* over a range of constant temperatures. The hybrids were less variable between temperatures than the parents, and they concluded that *Arabidopsis thaliana* performs no differently than an out-breeding organism. Kohel and White (1963) analyzed the variability of five parents and their F_1 progenies in *Gossypium hirsutum* L. The results though variable, indicated that the parents exhibited a greater degree of stability than the F_1.

MATERIALS AND METHODS

The experimental design consisted of parental plants and F_1 progenies in a nine-parent, complete diallel crossing system.

Plant Material

Parental lines derived from doubled haploids were used for eight of the parents and a highly inbred line was used for the ninth parent in this study to maximize homozygosity of the parents and thus improve the ability to discriminate beween

stability of parents and hybrids, if differences actually exist. These nine parental lines, which represent highly homozygous material from diverse backgrounds, are named and identified as follows:

(1) *Z104*—traces to a haploid plant in an irradiated stock of 'Rogers Acala.' This line was in its third generation of selfing following the doubling of the chromosomes of the haploid.

(2) *Z2557*—obtained from spontaneously doubled sectors of a naturally occurring haploid plant of the stormproof boll type in the breeding nursery at Lubbock, Texas.

(3) *Z112*—produced by doubling a haploid found in a stock whose complex pedigree of inter-strain hybrids involved crosses of strains of Rogers Acala. This line was in its second generation of selfing.

(4) *Glandless*—a line in its fifth generation of selfing following the doubling of a haploid plant found in a glandless $(gl_2gl_2gl_3gl_3)$ stock of 'Hopi' $\times$ 'Acala.'

(5) *A4-42*—a self-fertilized line from a doubled haploid plant found in 'A4-42.'

(6) *Empire*—a self-fertilized line from a doubled haploid plant of 'Empire.'

(7) *Stardel*—obtained from a spontaneously doubled sector of a naturally occurring haploid plant of 'Stardel.'

(8) *Z2444*—produced on a doubled haploid plant from a stock exposed to irradiation at Bikini. The irradiated stock gave rise to a translocation and a duplication-deficiency, and this was crossed to a genetic marker stock. The haploid plant carried the genetic markers: Okra leaf, L^o, Red plant body, R_1, Brown lint, Lc_1, and Naked seed, N.

(9) *TM 1*—a line derived from 'Deltapine 14' and propagated by pedigree selfing for 17 generations.

Characters Measured

Five characters were measured in both years of the experiment:

(1) *Plant height* was chosen as a measure of plant development because plants of *G. hirsutum* have an indeterminate fruiting habit and their gross reproductive capacity depends on total plant growth. An attempt was made to characterize plant development two-dimensionally and three-dimensionally by measuring the average spread of the side branches (width), in addition to measuring height. However, accurate width measurements were difficult to obtain, even on space-planted plants; and the resulting values failed to distinguish among entries and were considered no more informative than measurements of height alone.

(2) *Earliness* is expressed as the percent of the total seed cotton harvest on the first of three pickings and was measured because it is influenced by intraseasonal environmental fluctuations. Stability in earliness could reflect the ability of the plants to buffer against such fluctuations; however, cotton plants under certain amounts of stress shed young fruit forms and more mature fruit forms may be shed under severe stress. The shedding mechanism tends to protect the more mature fruit and thus insures the reproductive capacity of the plant. The facility for shedding under stress may be considered a labile character that promotes stability of reproductive capacity. In this context, the lability of fruit shedding may be reflected in variability of earliness.

(3) *Seed index* is expressed as the weight, in grams of 100 seeds, and is a measure of seed size. Stability of this character should reflect buffering capacity in seed development.

(4) *Lint percentage* is the percentage of lint (fiber removed from seeds by the ginning process) in the fruit. Lint hairs (fibers) are elongated cells of the seed epidermis. Growth and development of the lint is dependent on the development of the seed (Kerr, 1966) which, in turn, is strongly influenced by environment.

(5) *Yield of lint* is a composite character that measures the gross reproductive performance of the plant. Its development, as with lint percentage, is so closely associated with seed development that reproductive stability should be reflected in stability of this character. Thus while it may be argued that yield of lint is not a fitness character, per se, it serves as a useful measure of reproductive stability in this experiment. It is possible that fiber development is more labile than seed development, and that yield variability may be the best measure of variability associated with seed development.

Design and Analysis

The experimental design consisted of 81 entries (36 F_1's, 36 reciprocal F_1's, and 9 parents) in five-plant plots, replicated five times. Seeds were planted in the greenhouse and the resulting plants were then transplanted to the field nursery in rows spaced 100 cm apart with 90 cm spacing within the row. The design and procedure were followed for two consecutive years.

The within-plot variance of each entry for each character was computed. A logarithmic transformation of the variances was made, and the transformed data were analyzed by the analysis-of-variance procedure (Scheffé, 1959).

RESULTS AND DISCUSSION

Significance tests from the analysis of variance are presented in Table 1. There were significant differences among entries for height, lint percentage, and yield. The years $\times$ entries interaction was not significant for any character. This is especially noteworthy because it shows the same relative uniformity of performance of the entries in the two years of the experiment. The differences between F_1's and parents, and among arrays, were tested and the mean transformed variances of the parents and F_1's for each character are presented in Table 2. The parents were less variable than the hybrids in height, seed index, and yield, but significantly so only in the case of yield. The hybrids were less variable in lint percentage and earliness, significantly so only for lint percentage. The significant differences among arrays for height, lint percentage, and yield indicated that genotypic differences were important in determining variability.

The findings of this study were similar to those of Kohel and White (1963) in that no clear-cut distinction emerged between the variability of the parents and the hybrids even though the parents were less variable for three of the five characters measured. The choice of material derived from doubled haploids and the additional data provided by conducting the experiment over two years should have more sensitively measured differences between parents and their hybrids.

The basic premise in initiating this and the previous study (Kohel and White, 1963) was that suggested by Donald (1948), which stated that departures from the normal breeding system will reduce the stability of the developmental processes. This concept was developed further by Lerner (1954) in his discussion on homeostasis. Lerner's thesis was that outbreeding or-

Table 1. Significance tests for the analysis of variance of log (variance) for five characters.

Source	df	Mean squares				
		Height	Earli-ness	Seed index	Lint percentage	Lint yield
Years	1	0.01	0.021*	2.35**	30.59**	108.81**
Reps(within years)	8	0.43	0.002	0.19	0.45	1.15
Entries	80	0.23*	0.001	0.07	0.38**	0.33**
F_1 vs. F_0	1	0.22	---	---	0.78*	9.54**
Arrays	8	0.42*	---	---	1.32**	0.61**
E $\times$ Y	80	0.14	0.001	0.07	0.12	0.15
E $\times$ R (within years)	640	0.17	0.001	0.06	0.15	0.16
Total	809					

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

Table 2. Mean values (log variance) of the parents and hybrids for the five characters measured.

	Height	Earliness	Seed Index	Lint percentage*	Lint yield**
Parents	2.19	.05	.47	1.05	2.94
Hybrids	2.23	.04	.48	.99	3.19

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

ganisms had evolved a homeostatic mechanism associated with heterozygosity. The experimental evidence supports this thesis.

The converse would argue that inbreeding organisms have evolved homeostatic mechanisms associated with homozygosity. The experimental evidence is not clearly in support of this point. Few studies reported in the literature have dealt with this subject, and those that have been reported have not been decisive. Griffing and Langridge (1963) using an obligate self-fertilizing organism, demonstrated greater stability of heterozygotes over a wide range of constant temperatures, but they demonstrated greater homozygote stability within the constant temperatures in the few examples given. Stebbins (1950, 1957) contends that inbreeders have evolved from outbreeders and I agree. Also I believe that when we apply the converse of the homeostasis concept (as it is observed in out-breeding organisms) to inbreeding organisms, we are trying to place all organisms at the extreme poles of what is in fact a continuous spectrum. The greatest departure from an outbreeding system occurs when an organism can tolerate continued self-fertilization without losses of fitness (vigor), which results in the exposure and subsequent loss of sheltering deleterious genes and the fixation, or selection against and loss of linkage combinations that were maintained in heterozygous conditions.

The departure from predominant cross-fertilization to the tolerance of self-fertilization may also result in the loss of some of the unique heterotic and homeostatic properties associated with heterozygosity. It would be difficult to imagine that heterozygous organisms would ever be adapted to a lesser range of environments than homozygous organisms, but with the evolution of self-fertilization, heterozygotes per se may well become less fit, or at least have no advantage to particular environments. The heterozygosity that results from occasional outcrossing would provide sources of variability in a population; but as the organism evolved a system of obligate self-fertilization, it might evolve a distinct system of homeostatic homozygosity. The observations of Griffing and Langridge (1963) that heterozygotes were more stable over a wide range of constant-temperatures may not be too surprising, but it may be significant that the homozygotes were more stable within constant temperatures than the heterozygotes, in the limited sample shown. Stebbins (1950) suggests that one of the circumstances leading to self-fertilization is the adaptation of organisms to environments with uniform growing seasons and large interseasonal variation.

The cotton plant can naturally cross-fertilize, but it has relatively heavy pollen grains that are transported only by insect vectors. Many of the important pollen vectors have only a short foraging range, and are inefficient pollinators. The cotton plant has perfect flowers that readily self-pollinate, so foreign pollen has to compete with that already present. In a study of cross pollination of a complete male sterile, Kohel and Richmond (1962) found that, in the absence of self-pollen, insect pollination resulted in partial seed set of the individual bolls. This result reflected the ineffectiveness of insect cross pollination.

In addition, G. hirsutum in the wild is found in relatively isolated geographic areas. G. hirsutum has separated into several geographic races with fairly widespread distribution, but even within each group distribution is discontinuous. Short-range pollen distribution and geographic isolation favor inbreeding. Furthermore, G. hirsutum is an allotetraploid and is able to maintain residual inter-genomic heterozygosity even under conditions of inbreeding.

I am interested in the application of information about genetic systems to plant breeding techniques, and this study was concerned with cultivated G. hirsutum. We are handicapped in not being able to work with the more primitive forms of G. hirsutum, because they are short-day photoperiodic plants. The rather rapid transition from the short-day to a day-neutral form, which is cultivated as an annual, together with selection for other agronomically important traits, resulted in the loss of some of the more obvious adaptive traits. However, the basic adaptive fitness mechanisms cannot be expected to have been negated in this short evolutionary period.

CONCLUSIONS

The results of this study agree with those of Kohel and White (1963) in that there is no developmental homeostasis associated with F_1 hybrids, but neither is there pronounced stability associated with homozygosity. However, the data do show a tendency for greater stability of the parents. Results thus indicate that developmental homeostasis may not exist in cotton and, perhaps, other self-pollinated plants as it does in animals and in cross-pollinated species. The transition in natural breeding sysems from cross-fertilization to self-fertilization could cause the replacement of heterozygote advantage with a homozygote advantage. Developmental homeostasis of homozygotes may not have had time to develop in the organisms studied; or it may require more rigid maintenance of homozygosity and would become established only in obligate self-fertilizing species, or in those that approach obligate self-fertilization.

Cotton readily self-fertilizes, but it does not approach obligate self-fertilization. Perhaps most important is that cotton is an allotetraploid, and it maintains a form of heterozygosity between homeologous genomes. This would have the effect of increasing the time required for developing a truly homozygous state. The influence of the duplicated genetic information of the allotetraploid was demonstrated experimentally when Young and Murray (1966) showed that cultivated allotetraploid cotton (G. hirsutum) displayed less inbreeding depression and heterosis than cultivated diploid cotton (G. arboreum).

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