THE GENETICS OF FLOWERING RESPONSE IN COTTON. IV. QUANTITATIVE ANALYSIS OF PHOTOPERIODISM OF TEXAS 86, GOSSYPIUM HIRSUTUM RACE LATIFOLIUM, IN A CROSS WITH AN INBRED LINE OF CULTIVATED AMERICAN UPLAND COTTON[1]

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THIS paper, the fourth in a series reporting the research on flowering response of cotton at College Station, Texas, presents the results of the quantitative analysis of an experimental strain of American Upland cotton, *Gossypium hirsutum* L. race *latifolium*, designated as Texas 86.

Previous studies: In the first paper of this series, LEWIS and RICHMOND (1957) reported observing three factors which were important in controlling the time of flowering in the cross involving *G. hirsutum* race *marie-galante*, a short-day photoperiodic perennial cotton. According to them the short-day photoperiodic response was under multigenic control, and, in addition to photoperiodic response, *marie-galante* carried factors for delayed flower initiation. Also pronounced facultative shedding of fruit forms occurred under field-grown conditions.

In the second paper, (LEWIS and RICHMOND 1960), another allotetraploid species of cotton, *G. barbadense*, was studied, and the photoperiodic response was found to be under monogenic control. The day-neutral response was recessive, and there was no pronounced shedding of fruit forms or a delayed flowering response.

The third paper, (WADDLE, LEWIS, and RICHMOND 1961), reported studies of two selections of the race *latifolium*. The *latifolium* cottons are classified as annuals, and in contrast to experience with perennial *marie-galante*, no expression of lateness was found. At College Station, Texas they behaved similarly to *marie-galante* in that the photoperiodic response was under multigenic control and facultative shedding of fruit forms was pronounced. One of the *latifolium* parents was grown the same year at five widely separated stations in the U. S. Cotton Belt. Modification of the response to short-day photoperiod, by temperature and perhaps other factors, was demonstrated by the fact that: none of the *latifolium* parental plants flowered at College Station, Texas; all flowered at Shafter, California; and flowering at Lubbock, Texas, Sacaton, Arizona, and Brawley, California was late and sporadic.

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MATERIALS AND METHODS

This study utilized the F_2 and F_3 generations of a cross between Texas 86 and D&PL-14. Texas 86 is one of a number of stocks collected in the subtropics of North America which do not flower during the normal growing season at College Station; and it is of special interest to the research program, because it also carries genes for complete male sterility (RICHMOND and KOHEL 1961). Preliminary observations indicated that photoperiodism was quantitatively inherited in Texas 86. D&PL-14, an inbred line derived from a cultivated variety of American Upland cotton, is the stock used as the day-neutral parent in the first and third experiments of this series (LEWIS and RICHMOND 1957; WADDLE *et al.* 1961).

Forty-two F_2 plants of the Texas 86 × D&PL-14 cross were grown under a natural short-day regime in the greenhouse during the winter of 1960–61, and self-pollinated seeds were obtained from each plant to grow F_3 progenies during the forthcoming summer. Twenty seeds from each F_2 plant were planted in ten-inch pots in the greenhouse the first part of June. At the same time the F_2 plants growing in the greenhouse were cut back with the expectation that their regrowth would parallel the growth of the F_3 plants and that flowering would occur in approximately the same photoperiod. Because of failure to obtain complete stands, certain of the F_3 progenies were discarded; only the 30 progenies with eight or more plants were used in the present analysis. The mean F_3 progeny size was 13.6 plants.

The planting of the F_3 progenies some 45 days after the normal April 15 planting date for commercial cotton forced the fruiting period of the material into decreasing day-lengths. The total growing period was shorter but the effective flowering period was as long or longer than those of previous experiments. According to the chart given by LEWIS and RICHMOND (Figure 1, page 502, 1957), the longest day of the year occurs about June 15, when the daylight period is 14½ hours; at the time this experiment was terminated it was approximately 12½ hours.

A useful scale or type of notation is a prime consideration in an analysis such as the one undertaken here in which segregation in both the F_2 and F_3 populations included not only the major categories of flowering *vs.* nonflowering but the time of onset of flowering within the flowering group. A simple two-category classification in which each plant was scored as flowering or nonflowering would have provided some information for an analysis of the major flowering responses within each generation. The percent of nonflowering plants could be calculated for the F_2 population and each F_3 progeny; however, this two-class method of scoring would not discriminate among the plants that flowered at different times or permit critical statistical comparisons between F_2 parental plants and their F_3 progenies.

In the previous experiments of this series each plant that flowered was given a numerical value which corresponded to the number of days from planting to flowering; plants that did not flower were identified by an appropriate verbal notation. In the present study the base date for the numerical scoring was estab-

lished on the day the first flower appeared on a plant in the experiment (August 4, 1961) and each successive day of a 35-day scoring period was numbered consecutively. Thus, plants that flowered on day "1" were scored *1*, those that flowered on the next day were scored *2* and so on through *35*. A 35-day period of scoring for flowering response was established because previous research on flowering response in the field at College Station showed that plants which do not flower within approximately one month after the first flower occurs on a plant in the population usually do not flower before the field tests are terminated. To quantitate the nonflowering plants and to separate them from the flowering plants on the scoring scale, the plants that did not flower within the 35-day period arbitrarily were assigned a flowering score of *40*. Each plant in the F_3 progenies as well as each ratooned F_2 plant was scored for flowering response as just described.

RESULTS

The mean flowering score for each F_3 progeny was correlated with the percent of nonflowering plants in each F_3 progeny to determine how well the date of flowering score represented the flowering response. (The observed values are given in Table 1). The correlation was $r = .95$ for 28 df. This highly significant value indicated that the results obtained by either method of measurement should lead to approximately the same interpretation of photoperiodic response.

Since LEWIS and RICHMOND (1957) had found that factors controlling delayed flower initiation were present in *marie-galante*, the possibility that the flowering score was measuring genetic lateness in addition to photoperiodic response was investigated in the present material by cutting back 74 F_3 plants which had flowered during the summer, allowing them to flower again, and scoring them in the winter under a short-day regime, thus eliminating any response due to segregation for photoperiodism. The correlation of date of first flower in the summer with that in the winter was $r = .27$. This value is significantly different from zero at the $P = .05$ level, but is not significant at the $P = .01$ level. A certain degree of association was expected because these measurements were taken from the same plants in the same greenhouse pots; thus many of the environmental influences other than photoperiod were held constant. The value obtained when the correlation coefficient was squared ($r^2 = .07$) indicates that only seven percent of the variation in date of flowering can be attributed to similar responses in date of flowering under the two photoperiods. Obviously, the date-of-flowering score obtained in the summer was not confounded with estimates of early or late maturity.

These preliminary evaluations show that the mean score for date of flowering was highly correlated with the frequency of flowering and that it did not measure maturity. Therefore, date of flowering could be considered to be the phenotypic expression of the genotype reacting to a changing environment, and as such, it was a measure of the photoperiodic response of the plant. The numerical flower-scoring method was used in taking the data from which the following analyses were made. It must be remembered that a flowering score of 40 was assigned

TABLE 1

Flowering scores for the F_2 plants and F_3 progeny means and the percent of nonflowering plants in each F_3 progeny

Family no.	Flowering score*		Percent nonflowering plants
	F_2 plant	F_3 progeny mean	F_3 progeny
1	14	23	30
2	34	28	43
3	40	33	55
4	35	39	86
5	40	40	100
6	16	35	67
7	40	40	100
8	24	31	56
9	11	35	69
10	9	20	36
11	40	40	100
12	3	19	16
13	31	38	90
14	3	29	55
15	17	35	80
16	40	38	92
17	40	39	95
18	2	7	5
19	30	23	42
20	10	33	71
21	29	30	50
22	21	28	60
23	21	12	10
24	40	31	71
25	25	34	73
26	21	20	12
27	23	38	85
28	12	31	50
29	12	36	63
30	35	40	100
Mean	24	29	62

* Nonflowering plants were assigned a value of 40.

arbitrarily to plants in the nonflowering class. The arbitrary substitution of a value is not recommended as a procedure to be followed without recognizing that some of the assumptions associated with the calculation of variance estimates are being violated. However, in this case substitution makes it possible to analyze data which otherwise could not be subjected to statistical analysis. The writers take the position that the gain in genetic information outweighed the loss in statistical precision. Because this experiment was not replicated and homozygous parental and F_1 material were not included, it was not possible to test the validity of the statistical assumptions or to derive standard errors for estimated components of variance. These limitations must be recognized and considered when the

results are interpreted and in any attempt to extrapolate beyond the data obtained in this experiment.

The first presentation of the genetic components of variation associated with F_2 and F_3 generations was made by FISHER, IMMER and TEDIN (1932). MATHER (1949) elaborated on this earlier work, and his procedures and symbols were used in the subsequent analyses of flowering data taken on plants in the F_2 and F_3 generations of the Texas 86 × D&PL-14 cross. The following list gives the variances and covariances calculated:

Sources	Symbol	Components of variability	Observed values
Variance of the F_2	V_{F_2}	$D/2 + H/4 + E_1$	163.10
Variance of the F_3 progeny means	$V_{\bar{F}_3}$	$D/2 + H/16 + E_2$	74.55
Mean variance of F_3 progenies	$\overline{V}_{F_3}$	$D/4 + H/8 + E_3$	108.54
Covariance of F_3 progeny means and F_2	W_{F_2/F_3}	$D/2 + H/8$	62.72

D is the variation due to additive genetic effects, H is the variation due to dominance deviations, and E_1, E_2, and E_3 are the variations due to environmental factors associated with their respective variance estimates. This is a simple genetic model in which linkage, epistasis, and environmental-genotypic biases are assumed to be absent. The assumption of no environmental-genotype interaction seems presumptuous when it is known that genotypic variation was estimated as a result of the natural variation in the environment. However, the environment was identical for both generations and it was assumed that the genotypes respond in a similar additive manner to the environment.

Least squares estimates of the variance components were calculated from the observed variances by the solution of simultaneous equations. The least squares estimates were as follows:

$$\begin{aligned} D &= 118.46 \\ H &= 27.80 \\ E_1 &= 96.92 \\ E_2 &= 13.58 \\ E_3 &= 75.44. \end{aligned}$$

The average degree of dominance was calculated from the data given above. The ratio $\sqrt{\frac{H}{D}}$ is an estimate of the average degree of dominance considered over all loci. According to MATHER a value of 0.0 means no dominance and 1.0 shows complete dominance. The estimate in this experiment was 0.48 which indicated a partial expression of dominance. Determination of the direction of dominance was made by inspection of the F_2 and F_3 means. The average flowering scores

were 24 and 29 for the F_2 and F_3, respectively, which indicated that flowering was partially dominant over nonflowering.

For those who prefer to think in terms of heritability estimates, the two ratios $\frac{D}{D+H+E}$ and $\frac{D+H}{D+H+E}$, which are heritabilities in the narrow and broad sense, respectively, were calculated. The mean value for the three estimates of E, 61.98, was used; the estimates of heritability were .57 and .70 for the narrow and broad sense, respectively.

DISCUSSION

The study of flowering response in cotton reported in this paper, as well as in the others in this series of necessity, has been conducted without the benefit of facilities for critical control of the environment. However, the conditions under which these studies were conducted are similar to those an agronomist or plant breeder would encounter in the field when working with photoperiodic races in the temperate zone and for this reason the results have direct value in application. The previous studies relied on the natural environment of the field and populations of distinctive genotypic constitutions (F_0, F_1, F_2, F_3, and BC) for determinations of the inheritance of photoperiodic response. As a result, plants in the previous experiments were limited in the range of photoperiod utilized and were subjected to the natural seasonal variations in temperature and moisture. By growing the plants of this experiment in the relatively stable conditions of the greenhouse and timing propagating operations and plant growth in such a way that the initiation of flowering would occur at maximum day-length and continue through a decreasing day-length and finally by extending growth and fruiting beyond the period in the summer than possible under field conditions, the environmental range to which plants of specific populations or generations were exposed was much greater than that possible under normal field conditions. At the same time, the photoperiod, as well as the temperature and moisture conditions, was under more precise control.

In the material studied in this experiment and in the previous studies of *G. hirsutum*, flowering response was under multigenic control. However, phenotypic variability was detectable only in response to a changing photoperiod. LEWIS and RICHMOND (1957) discussed the possibility that a certain genetic or physiologic threshold must be reached before flowering can take place in a cotton plant. The results of their experiment and that of WADDLE *et al.* (1961) were in agreement with this concept. In all probability an underlying genotypic continuity characteristic of polygenic inheritance is operating but under a given environment there is phenotypic discontinuity associated with a threshold character: e.g., in this experiment, flowering *vs.* nonflowering. This threshold can be thought of in terms of a concentration of some flower-inducing substance or a rate at which this substance is produced. Whatever the mechanism, there is a threshold limit for each genotypic and environmental condition. This was the thinking underlying the design of this experiment and the interpretation of the results. This experiment was executed to take advantage of natural changes in

photoperiod progressively to allow phenotypic expression of the different genotypes present.

In a genetic experiment of this type, the choice of scale can greatly influence observed dominance. Partial dominance for flowering during long day-lengths was detected. The arbitrary selection of a flowering score of 40 to represent non-flowering types certainly may have introduced a bias. And as was discussed earlier, the scaling of the flowering score may have been such that it was not a normally distributed variable and thus may have introduced a bias. Another factor that should not be ignored is that of distribution of day-lengths which might influence the genetic variances of photoperiodism. A final factor that must be considered is the stage of growth of the two generations. Every effort was made to synchronize the growth of the F_2 and F_3 plants, but the fact remains that F_2 plants were represented by new growth from old wood and the F_3 plants were grown from seed.

No matter how troublesome or how they may have influenced plant growth and flowering response, the sources of bias just mentioned are inherent and unavoidable in an experiment of this nature. Interpretation of gene action by necessity is limited to the experimental material and environmental conditions of the experiment from which the data were taken. The important conclusions to be drawn are that photoperiodic response in *G. hirsutum* is multigenic, and that the *latifoliums* studied in this and other experiments in this series appear to display dominance for flowering under long-day photoperiods.

The results of the studies of flowering control in day-length-sensitive types of *G. hirsutum* have been in keeping with logical expectations when their natural habitat is taken into consideration. Under their native environment, seed production occurs in the winter season which is characterized by short-day photoperiods, cool temperatures, and drought. As found in these experiments, (1) short-day photoperiods are necessary for the induction of flowering, (2) cool temperature enhances flowering, (3) moisture or some associated factor promotes facultative shedding of fruit forms, and (4) those cottons which were classified as perennial may carry factors for lateness.

Generally in the evolutionary process the wild type is the dominant genotype. This was apparently not the case in *G. hirsutum* but it was quite true in *G. barbadense*. Flowering behavior of *G. hirsutum* is peculiar because in the wild forms there is a day-length-oriented mechanism for controlling flowering but in the cultivated forms such a mechanism apparently is lacking. Within the range of environments studied in this series of expeirments, the environments to which the cultivated cottons were exposed had no effect on their fruiting behavior. The cultivated forms therefore may be said to be typified by having no control mechanism for response to the wide variation in day length that occurs annually in the temperate zone.

SUMMARY

F_2 and F_3 populations of Texas 86, *Gossypium hirsutum* race *latifolium*, × D&PL-14, an inbred line of cultivated American Upland cotton, segregating for

short-day photoperiodism were grown in the summer greenhouse and scored for date of flowering. Flowering scores were tested and shown to represent plant response to photoperiod; i.e., they were free from any bias measuring maturity. These flowering scores were used to calculate the variance estimates of the genetic components; and the analysis demonstrated that flowering, which is quantitatively inherited, was partially dominant over nonflowering. The dominance ratio was 0.48.

These results were discussed in connection with the findings reported in previous papers of this series, and the consistency of all findings was emphasized. Further, attempts were made to relate experimental findings to the environmental conditions of the wild cotton populations.

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