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Cytoplasmically Controlled Male Sterility in Cotton

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IN THE ideal case of cytoplasmic male sterility, transfer of the genome of one species into the cytoplasm of another should result in 100% male sterility, while the same genome should be 100% fertile in its own cytoplasm. A dominant, non-deleterious gene should be available to restore 100% fertility when it is heterozygous in the genome introduced into the foreign cytoplasm. The ideal situation has not yet developed for cotton.

The interspecific property transfer program for cotton was begun at Stoneville, Mississippi, in 1948. One of its objectives was development of cytoplasmic male sterility. After 14 years and several thousand progeny rows, the first consistent pattern of the interactions governing male sterility in this material began to come into focus. It was evident that male sterility expression was most complex, and that it involved cytoplasm, genotype, and environment. To clarify this situation, a detailed study was made. Utilized as parents were: *(1)* a single, true-breeding, essentially male sterile plant with cytoplasm from a diploid species, and *(2)* a fertile Upland doubled haploid. Our report deals with male sterility[3] as it occurs within the descendants of these two plants. This is not the only sort of sterility available from the derivatives of the interspecific hybrids developed and studied at Stoneville, Mississippi, nor is it the most complete sterility. However, it is the first cytoplasmically controlled sterility of cotton to be intensively studied and to have its gene-cytoplasm-environment interactions worked out. The general pattern of these interactions holds for a very wide range of progenies from crosses which involved other parents of similar origin, but most of them differ in detail from the single family of plants described in this report.

MATERIALS AND METHODS

The essentially male-sterile parent plant used for this study had cytoplasm from *Gossypium anomalum* Tod., a wild, lintless, diploid species from Africa. A hybrid was produced between *G. anomalum* and *G. thurberi* Wawra & Peyr., a wild, lintless, diploid species from Arizona. The chromosome number of the hybrid was doubled; the resulting amphidiploid was crossed with *G. hirsutum* L., the tetraploid, linted American species usually grown for commercial production of fiber. A doubled haploid of Upland cotton, 'M8,' was used as the *G. hirsutum* parent. A plant breeding true for high sterility resulted from three backcrosses of selected, partially sterile plants to 'M8,' plus two generations of selfing. All of the progeny rows reported in this paper are descended from 'M8' and 1961:999–8; the latter plant is hereafter designated as 'C9.' Sterile plants and their F_1 hybrids with 'M8' cytoplasm were brought into the greenhouse from the field in the fall of 1962 for producing test-crosses not already available from field plants. Two sets of progenies were grown: parents selfed, F_1, F_2, backcross to 'M8,' and backcross to 'C9;' one set had *G. anomalum,* the other *G. hirsutum* cytoplasm (Figure 1).

Flowers were scored by a modification of the Justus–Leinweber[4] system. A score of "0" denotes a flower with no fertile anthers, "1", up to 25% fertile anthers, "2" denotes 25–75%, and "4" 100% fertile anthers. The number of flowers in each class on each date was recorded on printed tags tied to the plants. The tags were divided into columns for each score and rows for each date; a ticket-punch was the most rapid means for recording the number of flowers of each class on each date when the flowers were checked for sterile anthers; on days when a single plant had 10 or more flowers, numbers were written in the appropriate rectangles with laundry-marking pens.

Under most environmental conditions plant classifications were reliable indications of genotype. Plants used in this study were classified for sterility by either or both of two criteria: *(1)* a mean sterility score determined from all flower scores recorded for the plant, sometimes based on over 100 flowers; and *(2)* a classification as "sterile", "intermediate", or "fertile" on the basis of the distribution of flower scores among the 5 possible classes. The second method proved as trustworthy as the first in 1962 and 1963, and it had the advantage of being suited for rapid field determination by a glance at the plant tag, even before the plant had finished flowering. A plant was classified "sterile" if no more than 1 of 10 or more flowers scored over a period of 3 weeks or more was a "3" or "4"; a "fertile" plant had no more than one "0" or "1" score flower; an 'intermediate" usually had at least 1 flower in each of the 5 possible classes, frequently within a period of 2 or 3 days. The 1964 season had unusually variable weather conditions, and the first system had to be used for classifying plants.

For some purposes, such as measuring the effect of environmental variations, or for comparing relative sterility levels of homogeneous rows, the daily mean sterility scores for all flowers in a progeny row proved useful. For example, when it became apparent that the dense stand and rank growth of some of our 1963 rows with only "fertile" plants were causing us to use a great deal of time and effort to determine which plant produced each highly fertile flower, we decided to simply count the total flowers in each class, and to record the numbers each day on tags at the end of each row.

Because variances for flower score data were dissimilar for reciprocal hybrids, Chi-square techniques were used for most of the statistical treatments of the data.[5]

RESULTS AND CONCLUSIONS

The studies reported in this paper began with an attempt to determine whether or not there were significant differences in sterility between reciprocal crosses of plants with cytoplasm from different species. Frequency distributions

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[3] Throughout this paper, the term "sterility" refers to male sterility, unless there is a specific statement to the contrary.

[4] Justus, Norman, and C. L. Leinweber. 1960. A heritable partially male-sterile character in cotton. J. Hered. 51:191–192.

[5] We wish to thank Dr. Walter Drapala, of the Mississippi Agricultural Experiment Station, State College, Mississippi, for his helpful suggestions for handling these data.

by mean plant sterility scores, graphs of mean daily sterility scores of progeny rows, and plant classification counts within progeny rows all demonstrate that selfing 'M8' produces only "fertile" plants, and that these plants remain highly fertile under summer weather conditions normal for the Mississippi Delta (Figures 1B and 2). Conversely, when 'C9' is selfed, all of the plants are of "sterile" classification, and they remain highly sterile throughout a wide range of environmental fluctuations (Figures 1A and 2). During several seasons of growing selfed progenies neither of these parents has produced off-type plants, so far as fertility or sterility is concerned. Consequently, we feel justified in considering them homozygous for whatever genes determine their respective levels of anther fertility. Certation (selective fertilization), lethal factors, or differential survival of zygotes, even if they should occur, could not affect distribution of a gene present in every gamete produced by these plants. Table 1, Figure 2, and Figures 1D and 1E show highly significant differences between reciprocal F_1 hybrids produced by crossing 'M8' and 'C9.' The simplest explanation for these differences is that cytoplasm affects the expression of genes controlling anther development and differentiation.

By the end of 1961 it was obvious that environmental factors were important and would have to be considered in studying any aspect of male sterility of these and our other interspecific cotton hybrids. The general effect was that low fertility conditions increased the probability of flower scores at the lower end of the normal fertility range for a plant or group of plants, while the environmental conditions which caused high fertility increased the probability of flower scores at the upper end of the normal range. Most of the figures which plot mean sterility of progeny rows against time are from results for the year 1963, because that was the year when the critical testcrosses were grown. The only major difference of these curves from those of all of the other years when some similar progenies were grown on a smaller scale is that the dates on which the fluctuations occur vary from year to year. By 1964, seed was available from plants with *G. anomalum* cytoplasm, known to be homozygous "fertile" and homozygous "sterile." Figure 3 shows that there is a close negative correlation between the daily mean sterility scores for homozygous selfed progeny rows and their heterozygous F_1, and the daily maximum temperature 15 days before anthesis. The correlation coefficient of −0.51 was significant at the 0.05 level of probability for the "fertile" row. The values of −0.62 for the F_1 and −0.63 for the "sterile" rows were significant at the 0.01 level of probability. The fact that there were only a few days in 1963 when the maximum daily temperature was as high as 95° F. greatly simplified working out the genetic basis for the sterility of 'C9.'

After we had found that reciprocal F_1 hybrids between 'M8' and 'C9' differed significantly in sterility, the next logical step was to determine the number of genes with important effects on the development and release of fertile pollen by these plants. Even before 1963 it was apparent that most plants in rows segregating for sterility could be classified rather easily as "fertile," "intermediate," or "sterile," but that in some cases there was no clearly defined line of separation between the classes. Whatever the number of genes involved, backcrosses to both 'C9' and 'M8,'

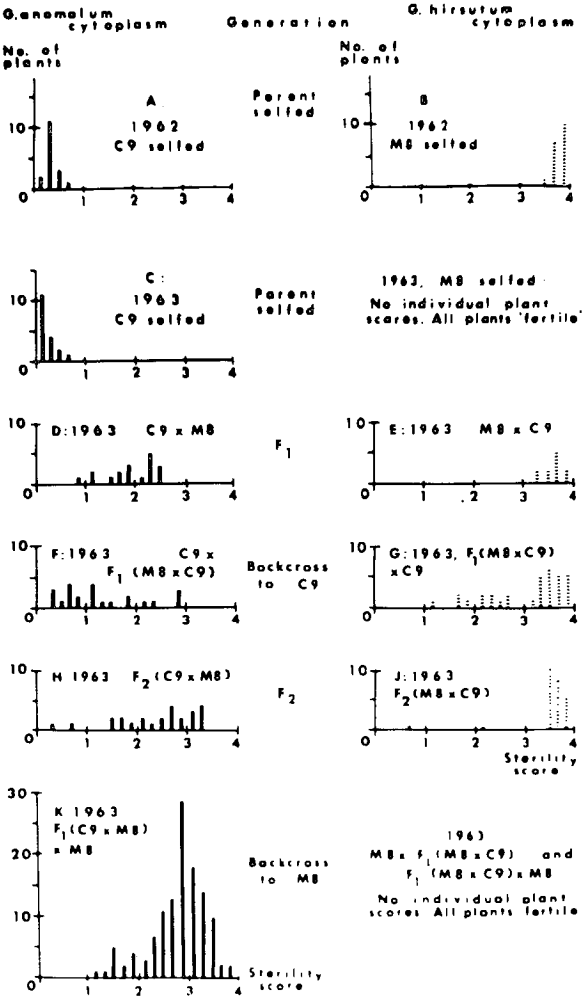

Figure 1. Comparison of frequency distributions for mean plant sterility scores of reciprocal hybrid progeny rows from crosses involving 'C9' ("sterile", *G. anomalum* cytoplasm) and 'M8' ("fertile", *G. hirsutum* cytoplasm).

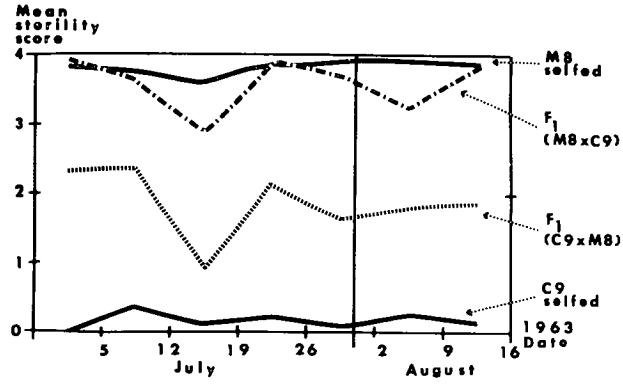

Figure 2. 1963: Mean weekly sterility scores for selfed progeny rows from *G. hirsutum* 'M8' and 'C9' (a "sterile" plant with *G. anomalum* cytoplasm) and their reciprocal F_1 hybrids.

Table 1. Number of flowers in 5 sterility classes from paired rows of reciprocal F_1 hybrids between 'C9' and 'M8', with Chi-square values testing the probability of a homogeneous distribution among the reciprocal hybrids.

Year	Cytoplasm	Number of flowers scored					Mean row score	Chi-square*
		0	1	2	3	4		
1962	G. anomalum	109	207	206	322	61	2.0	775
1962	G. hirsutum		1	10	277	422	3.6	
1963	G. anomalum	56	127	99	124	22	1.8	348
1963	G. hirsutum	1	4	4	61	155	3.6	

* For the 1962 reciprocal hybrids, there is less than a 0.01 probability of a Chi-square value above 9.21 from a random distribution of flower scores. For the 1963 hybrids the corresponding Chi-square value at the 0.01 level of probability is 6.63.

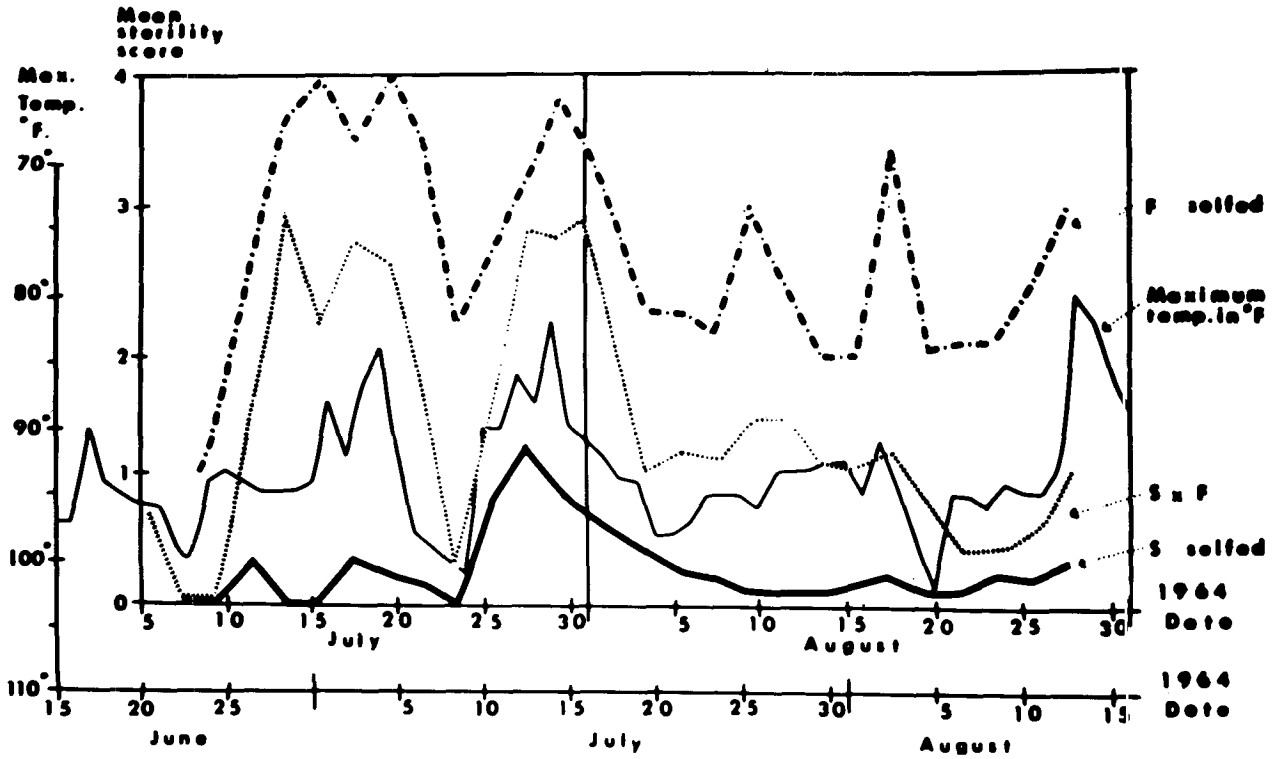

Figure 3. 1964: Inverse relation between the maximum daily temperature 15 days before anthesis and the mean daily flower scores of progeny rows from plants with *G. anomalum* cytoplasm which were classified "sterile" (S), and "fertile" (F) on the basis of flower scores in 1963.

Table 2. Number of plants classified "sterile" and "intermediate" in 5 backcross progeny rows with *G. anomalum* cytoplasm grown in 1963 from crosses of 'C9' × F_1 ('M8' × 'C9'), with Chi-square values testing a 1:1 segregation ratio.

Sterile parent	Number of plants classified		Chi-square	Probability of a larger Chi-square
	"sterile"	"intermediate"		
C9	12	11	.043	.90-.80
C9-1	6	9	.600	.50-.30
C9-2	8	9	.059	.90-.80
C9-3	7	5	.333	.70-.50
C9-4	7	10	.529	.50-.30
Total	40	44	.190	.70-.50

in both *G. anomalum* and *G. hirsutum* cytoplasms, should give twice as many plants homozygous for sterility or for fertility genes as could be obtained from the same number of plants in F_2 progeny rows. As in every other year when reciprocal F_1 hybrids between 'C9' and 'M8' have been grown, the row with *G. hirsutum* cytoplasm consisted only of highly fertile plants (Figures 1E and 2); in most of the flowers produced all of the 100-or-so anthers were fertile (Table 1). The F_1 row with *G. anomalum* cytoplasm consisted of plants producing flowers highly variable in sterility (Table 1), and all plants with enough flowers for reliable classification were "intermediate" (Figure 1D).

In the backcrosses to 'C9' with *G. anomalum* cytoplasm (Figure 1F), the separation between "sterile" and "intermediate" plants was clear-cut. Visitors to the field found it easy to classify the plants in the progeny rows listed in Table 2. For all five backcross progenies, the pollen was an F_1 plant with *G. hirsutum* cytoplasm, not only because pollen was more abundant, but also to determine whether or not sterility genes would be maintained unchanged in the heterozygous state in a "fertile" cytoplasm.

The reciprocal backcross to 'C9' with *G. hirsutum* cytoplasm also produced two classes of plants. The 22 plants classified "fertile" produced flowers very close to the 'M8' level of fertility (Figures 1G and 6), while the 12 plants classified "intermediate" were nearly as fertile except following a brief period of hot weather, when nearly all of the flowers they produced became partially or entirely sterile. The Chi-square value testing a 1:1 segregation ratio was 2.84 ($p = 0.10 - 0.50$) for this cross.

Table 3. Number of plants classified "intermediate" and "fertile" in 12 backcross progeny rows with *G. anomalum* cytoplasm grown in 1963 from crosses of F_1 ('C9' × 'M8') × 'M8', with Chi-square value testing a 1:1 segregation ratio.

Mean score of F_1 plant in 1962	Mean sterility of 1963 row	Number of plants classified		Chi-square	Probability of a larger Chi-square
		"intermediate"	"fertile"		
1.50	2.70	4	1		
1.98	1.83	1	1		
2.26	2.87	6	5		
2.56	2.16	6	5		
2.16	2.19	7	3		
2.88	3.04	3	6		
2.41	3.16	7	11		
1.40	2.45	8	7		
1.77	2.75	6	4		
1.42	2.92	2	2		
2.18	2.89	8	4		
2.03	2.83	8	7		
Total for 12 rows		66	56	0.82	.50-.25
Mean sterility for total		2.51	3.27		

Correlation between sterility of parent plant and mean sterility of progeny row = + .12 NS.

The backcross to 'M8' with *G. anomalum* cytoplasm produced only plants classified "intermediate" and "fertile." Table 3 presents the distribution of plant classifications within the 12 progeny rows grown from individual F_1 plants backcrossed to 'M8' in 1962.

The backcross to 'M8' with *G. hirsutum* cytoplasm produced only plants classified "fertile". All but 10 of over 450 flowers examined in the 2 progeny rows grown in 1963 were scored either "3" or "4", and no "0" score flowers were produced. All flowers in these 2 rows were checked on the same dates as for the other rows in the 1963 test block. For reasons discussed earlier, individual mean plant scores are not available for these rows. As shown in Figure 4, the daily mean sterility scores are very similar for 'M8' and for the backcrosses to 'M8' with *G. hirsutum* cytoplasm.

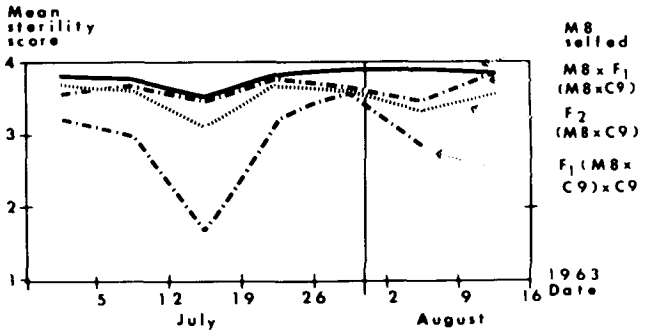

Figure 4. 1963: Mean weekly sterility scores of progeny rows with *G. hirsutum* cytoplasm, from 'M8' and F_1 ('M8' × 'C9').

The small F_2 populations segregated for sterility as one would have expected on the basis of the backcross data (Figures 1H and J); the row with *G. anomalum* cytoplasm produced plants classified "sterile," "intermediate," and "fertile," and the rows with *G. hirsutum* cytoplasm produced many "fertile" and a few "intermediate" plants.

Plant classifications on the basis of the distribution of the flower scores recorded tell us something about the similarities and differences in performance of plants as they grow in the row or in the field. If these classifications happen to be closely related to part of the genetic endowment of the plants, they can serve as a more-or-less- reliable means for determining that part of the plant's genotype. Of course, we have as yet no direct means of determining what genes are actually present within any one gamete or zygote. Classifying plants by probable genotype depends on: *(1)* determining the usual range of phenotypes for any one genotype or set of genotypes and basing a classification system on the probable phenotypes, *(2)* working out a hypothetical genetic system which could explain the distribution of classes which occur as the result of crossing phenotypes of known origin, *(3)* testing how well the observed distributions fit the theory, and *(4)* predicting the behavior of future generations from crossing plants in the various classes.

The sterility segregation obtained from selfs and crosses of 'M8' and 'C9' might be explained by the following theory: Regardless of the genotype involved, for any one genotype a higher level of fertility occurs within *G. hirsutum* than within *G. anomalum* cytoplasm, and this level of fertility is depressed to some extent whenever temperatures become extremely high. The 'M8' genotype ordinarily produces "fertile" plants with either cytoplasm, but under stress conditions this genotype in *G. anomalum* cytoplasm may produce enough partially or entirely sterile flowers so that plants will be classified "intermediate". The 'C9' genotype associated with *G. anomalum* cytoplasm produces sterile flowers unless field temperatures are abnormally low; only plants classified "sterile" have been produced by this gene-cytoplasm combination in the four years during which 'C9' has been grown. The 'C9' genotype in *G. hirsutum* cytoplasm is highly fertile except following periods of high temperature; such plants would usually be classified "intermediate", but some would be classified "fertile" if they did not produce flowers during periods of low fertility.

Heterozygous plants with *G. anomalum* cytoplasm are extremely sensitive to environmental fluctuations; such plants usually produce flowers of all five classes, and under almost any growing conditions they will be classified "intermediate". Heterozygotes with *G. hirsutum* cytoplasm are nearly as fertile as 'M8,' and all such plants would be classified "fertile".

If one allele not present in 'M8' and homozygous in 'C9' is the major agent interacting with a cytoplasmic structure to prevent anther fertility under most environmental conditions, we would expect some sort of 1:1 segregation ratio within backcross progenies, and a 1:2:1 in F_2 rows, with no sort of segregation by class within selfs or F_1 populations. Chi-square tests of the ratios obtained from the populations grown in the field during 1963 (Tables 2 and 3) indicate no significant deviations from the ratios expected on the basis of the one-gene theory. Within the populations with *G. anomalum* cytoplasm grown in 1963, the probability would appear high that a plant classified "sterile" is homozygous for the 'C9' gene for sterility, that a "fertile" plant is homozygous for its allele from 'M8,' and that an "intermediate" plant is heterozygous.

"Fertile," "sterile," and "intermediate" plants with *G. anomalum* cytoplasm were moved into the greenhouse from the field at the end of the 1963 season. These plants were used as parents for test-crosses to determine whether or not progeny rows would produce plants of the classes expected on the basis of one gene not present in 'M8.' As shown in Figure 3, temperatures were extremely variable during the 1964 season. Since the 'M8' genotype in *G. anomalum* cytoplasm produced numerous partially or entirely sterile flowers at some periods, with the result that all such plants were classified "intermediate" in 1964, no clear-cut separation of heterozyotes from homozygous fertile plants was possible. Nevertheless, both the distribution of "sterile" plants, and the mean plant sterility scores within all of the test-cross rows (Figure 5), still fit neatly into a "one-gene plus 'M8'" hypothesis.

The data available at present suggest that the sterility occurring in this group of plants is primarily due to one gene present in 'C9' and not present in 'M8;' that cytoplasm, gene dosage, and environment all affect expression of the gene; and that classification of plants according to the level of sterility is highly effective for separating the homozygous sterile from the other two genotypes, while its effectiveness for separating the heterozygous from the homozygous fertile plants with *G. anomalum* cytoplasm depends on the environment in which the plants happen to be grown.

DISCUSSION

The data now available support our working hypothesis that anther fertility depends upon a chemical process or series of processes within the developing anthers. The process involves a gene-product, a cytoplasmic structure, and some factor outside of the plant. Anther fertility could be affected by (a) regulating the amount of gene-product available, (b) changing the cytoplasmic surface on which it reacts, (c) altering the reaction rate, or (d) destroying the end-product of the reaction.

The original plants from the three species used to produce the material for this study all had abundant fertile anthers under virtually any environmental conditions that allowed flowering. Anther sterility differs from most properties transferred from wild species or hybrids to Upland cotton in that it is a property present in neither the wild nor the cultivated ancestral species. We can assume that natural selection is responsible for the presence within each species of the complementary genes and cytoplasm which cooperate to develop fertile anthers. The third back-

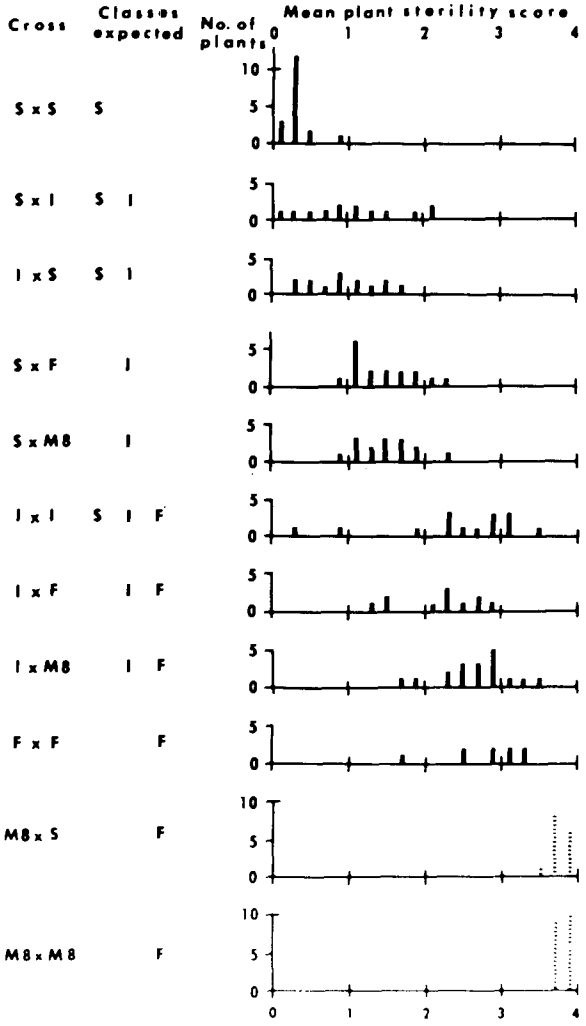

Figure 5. Relative frequencies of mean plant sterility scores for progeny rows from test crosses of related single plants classified "sterile" (S), "intermediate" (I), and "fertile" (F) on the basis of 1963 flower scores.

cross to 'M8' should have approximately 94% of the *G. hirsutum* genome. Consequently, it is not surprising that the differences between reciprocal hybrids show that fertility is much higher within *G. hirsutum* cytoplasm than for the identical genotype within *G. anomalum* cytoplasm.

Plants grown under field conditions are not well suited for supplying answers to the question of what sort of substance or process determines the level of anther fertility. If a gene product were not involved, the dosage effect indicated by different levels of fertility for homozygous and heterozygous sterile plants would not be apparent, nor would segregation ratios be obtained like those in Tables 1 to 3. However, within *G. hirsutum* cytoplasm the quantity of gene-product seems to be limiting only under certain conditions. Temperature, particularly maximum temperature about 15 days before anthesis, seems closely associated with the level of expression of the sterility gene. This does not exclude the possibility that some environmental factor other than heat may be active (Sarvella).[6] Under summer field conditions, high maximum and minimum daily temperatures, intense light, low soil moisture, and high evaporation rate occur together. Nevertheless, temperature is known to have important effects on both biological and inorganic chemical reaction rates. Goldschmidt's[7] discussion of temperature effects on mutant gene action in *Lymantria* and *Drosophila* applies very well in this case, if we add a limiting cytoplasmic effect. Perhaps we could assume that the gene for sterility slows, rather than stops, an essential process; that the end-product of this process is thermolabile; that *G. hirsutum* cytoplasm is so efficient, either in producing the substance or in using it, that available amount of the substance is seldom a limiting factor; and that a stage of anther differentiation which occurs approximately 15 days before anthesis is particularly sensitive to this substance.

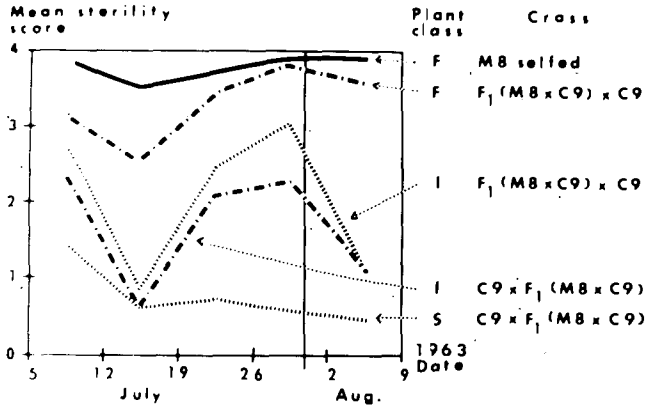

Figure 6. 1963: Comparison of mean weekly sterility scores for plants classified "sterile" (S), "intermediate" (I), and "fertile" (F) in reciprocal backcross rows of 'C9' (a "sterile" plant with *G. anomalum* cytoplasm) and F_1 ('M8' × 'C9') (a "fertile" plant with *G. hirsutum* cytoplasm).

Only a few of the over 20 species of *Gossypium* have as yet been used as sources of cytoplasm in a program to backcross the Upland genome into other cytoplasms. A considerably higher number of species has furnished genes for transferring properties from other species into Upland genomes and cytoplasm. Very few of the mutant genes which cause sterility in Upland and other cottons have been collected and studied genetically, even within the cytoplasms in which they occur. It seems probable that a systematic program which involved collecting as many cytoplasms and as many genes for sterility as possible, and then testing their effects in combination, could result in a wide assortment of complementary genes and cytoplasms which could produce any desired level of fertility under some particular set of environmental conditions.

SUMMARY

Virtually complete male sterility results when a partially recessive gene is homozygous in cotton plants with *G. anomalum* cytoplasm. Reciprocal crosses of these plants with *G. hirsutum* 'M8' produce highly fertile progenies if the cytoplasm is from *G. hirsutum* and partially sterile progenes if the cytoplasm is from *G. anomalum*. Environmental factors, of which temperature may be the most important, determine the level of expression of sterility within any one genotype-cytoplasm combination.

[6] Sarvella, Patricia. 1964. Variation of cytoplasmic male-sterile cotton with environment. Agron. Abs. 1964:78–79.

[7] Goldschmidt, Richard. 1938. Physiological Genetics. McGraw-Hill Book Co. New York.