LEAF LOBATION IN GOSSYPIUM AMPHIDIPLOIDS AS DETERMINED BY LEAF-SHAPE GENES OF GOSSYPIUM DIPLOID SPECIES[1]

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INVESTIGATION of leaf lobation, or leaf-shape expression, is informative about phenotypic expression and is suggestive of evolutionary change in diploid and amphidiploid species of Gossypium. The problem at hand is the relationship of leaf-shape genes of the amphidiploid species and the leaf-shape genes of the closely related species of the A and D diploid genomes, since the Gossypium amphidiploids 2 (AD) are considered to have originated from a hybrid between a species similar to diploids of the A genome and a species similar to diploids of the D genome (BEASLEY 1940; HUTCHINSON, SILOW, and STEPHENS 1947; SKOVSTED 1933; STEPHENS 1944).

A developmental study of leaf lobation made by STEPHENS 1945c indicated that the common leaf shapes of the amphidiploid species are determined by the same genes as the leaf shapes of the diploid species of the A genome. This similarity of genes for leaf-shape expression is to be expected according to the hypothesis of HUTCHINSON *et al.* (1947), that the amphidiploid species have a recent common origin from an A genome species, presumable *G. arboreum* L. $2A_2$, crossed with D genome *G. raimondii* Ulbr. $2D_5$. It would appear then that the alleles at a leaf-shape locus of the amphidiploids are the same as the genes of the leaf-shape locus of the A_2 diploid.

Another range in leaf lobation is found among the various species of the D diploids, each phenotype presumably being controlled by genes at a single locus (STEPHENS 1945c). GREEN (1953), working with a new leaf shape in amphidiploid *G. hirsutum* L. $2A_hD_h$, assumed that the gene had been transferred from *G. thurberi* Tod. $2D_1$ via the synthetic amphidiploid $2A_2D_1$. A genetic test showed the new gene to be an allele of the gene for okra-leaf shape in the amphidiploid, and GREEN thus presumed the leaf-shape locus was located on the D genomes of the amphidiploids. RHYNE (1951) showed that a lobed-leaf expression of *G. thurberi* segregated in progeny of the synthetic hexaploid hybrid, *G. hirsutum* × *G. thurberi* $2A_hD_hD_1$, as expected if the leaf-shape locus were located on the D genomes of the hybrid.

An investigation using transferred leaf-shape genes of the A and D diploids in Gossypium amphidiploids is needed. The relationships of the genes of the diploids

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and the naturally occurring amphidiploids should fall in certain predictable patterns:

1. If the Gossypium amphidiploids are new, i.e., if they have a recent and common origin, the leaf-shape genes of the diploids may be shown to be the same as genes of the amphidiploids. The criteria for judging if they are the same are: The transferred leaf-shape genes of the diploids produce phenotypes that are identical with those of the amphidiploids. The transferred genes have the same dominance and epistatic gene interaction that the genes of amphidiploids have. And genetic segregation in appropriately marked hybrids shows that the transferred genes are located either at the same locus or at different loci. When the genes are located at the same locus, they can be called "identical" genes, but when they are located at different loci, and each locus is in a different genome of the amphidiploid, they can be called "duplicated" genes. A new amphidiploid generally is expected to have some duplicated genes.

2. If the Gossypium amphidiploids are old species and/or if they do not have a common origin, the leaf-shape genes of the diploids may be shown to be different from the genes of the amphidiploids. The criteria for judging if they differ are: The transferred leaf-shape genes of the diploids produce phenotypes that differ from those of the amphidiploids. The transferred genes and the amphidiploid genes have different dominance and epistatic gene interactions, and genetic segregation in appropriately marked hybrids shows that the transferred genes are located at the same locus or at different loci. When the genes are located at the same locus, they can be called "multiple alleles." Multiple allelism has been demonstrated for a number of transferred diploid genes at other loci of the natural amphidiploids (GREEN 1953; STEPHENS 1945a,b,c; HUTCHINSON *et al.* 1947; RHYNE 1951, 1958). When the genes are located at different loci and each locus is in a different genome of the amphidiploids, they can be called "independent" genes.

According to the HUTCHINSON *et al.* (1947) hypothesis of a recent and common origin it would appear that transferred genes for leaf lobing from the A genome diploids would behave in amphidiploids as identical genes of the alleles at the leaf-shape locus. It is possible, however, that a transferred gene from one D diploid may be identical with the common leaf-shape allele of one of the natural amphidiploids and may be allelic with the common allele of another amphidiploid. Such evidence for multiple allelism would be suggestive either of divergence among amphidiploids or possibly of multiple origin of the amphidiploid species.

This paper is concerned with genetic behavior of leaf-shape genes from various A and D diploids when transferred to Gossypium amphidiploid background. Emphasis is placed on genetic segregation and leaf-shape expression after the gene transference has been accomplished. Anomalous behavior of genes is, but Mendelian inheritance is not reported for the transference procedure. Gene relationships are to be determined from segregation ratios in progeny of appropri-

ately marked hybrids and from dominance and epistatic gene action in particular genetic backgrounds.

MATERIALS AND METHODS

Leaf-shape phenotypes were observed in the hot, arid Arizona and in the humid North Carolina climates. These expressions were essentially the same as those reported by STEPHENS (1945c) for the tropical Trinidad location. Consequently, the expressions figured by STEPHENS (1945c) have been partly adapted to construct Figure 1. Only the extremes in leaf lobation have been selected from the various expressions found in either the A or D diploids; but the intermediate expressions were found to conform to these reported patterns.

The selected alleles from the A diploids were laciniate and Asiatic "broad". Laciniate is the dominant allele of this multiple allelic series (HUTCHINSON

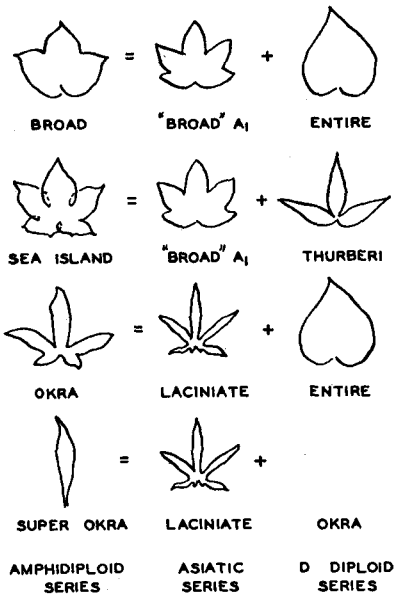

FIGURE 1.—Leaf lobation in amphidiploid and diploid Gossypium species. The column on the left contains the four common, leaf lobations and the alleles at a common locus of the natural amphidiploid species as they appear in Upland varieties of amphidiploid *G. hirsutum*. The center and right-hand columns show respectively the extremes of leaf-shape expression in the A and D diploid species. The amphidiploid phenotypes also could be produced by interaction of transferred, diploid, leaf-shape genes in the A and D genomes of the amphidiploids. For example, a broad leaf in the amphidiploids is the interaction product of Asiatic "broad" and D diploid entire genes, as is shown in the first row. Sea Island was the interaction of Asiatic "broad" and D diploid "thurberi", 2nd row. Okra-leaf shape was produced by Asiatic laciniate and D diploid entire, 3rd row. A super-okra phenotype could be produced by Asiatic laciniate and okra of the amphidiploid D genomes, 4th row. Okra-leaf shape is omitted from the right column of row 4 since it is not known in a D genome diploid. Okra and super okra are D genome alleles in the amphidiploids but they must be considered as evolution products of the amphidiploid species.

1934; STEPHENS 1945c) and Asiatic "broad" is the recessive. In Figure 1 both expressions are taken from *G. arboreum* $2A_2$, although the recessive form is commonly found in *G. herbaceum* L. $2A_1$. From the D diploids the extreme amount of leaf lobation is found in *G. thurberi* $2D_1$ which furnished the "thurberi" allele and the least amount, the entire (unlobed) leaf, is figured from *G. raimondii* $2D_5$.

Four alleles have been established in the natural amphidiploids (STEPHENS 1945a,c) ranging from a dominant strap-like leaf of super okra to a recessive broad-lobed expression. Any phenotype called broad refers hereafter to the recessive expression in the amphidiploids.

As a guide for the reader, Figure 1 shows the amphidiploid leaf shapes and the interactions of various diploid alleles that produce similar expressions in amphidiploid background. This procedure is necessary since a common amphidiploid phenotype will be shown to result from the interaction of more than one set of alleles. Figure 1 thus is a rearrangement of the figure and hypotheses of STEPHENS (1945c). The interaction of genes Asiatic "broad" and "entire" of the A and D diploid genomes, respectively, produces the broad-leaf expression of the amphidiploids. This conforms with the figure and hypothesis of STEPHENS. These expressions are found across the top row of Figure 1. The interaction of genes Asiatic "broad" and "thurberi" of the A and D diploid genomes, respectively, produces a lobed leaf. This phenotype is indistinguishable from the "Sea Island" expression in amphidiploid *G. barbadense* L. $2A_bD_b$. These are found in the second row from the top of Figure 1. The interaction of genes laciniate and recessive entire of the A and D diploid genomes, respectively, produces an okra expression in the amphidiploids. This conforms to the phenotype but not the location predicted by STEPHENS (1945c). These are found in the third row from the top of Figure 1. The super okra expression of the amphidiploids was produced by the interaction of genes laciniate and okra of the A and D genomes, respectively, of amphidiploid *G. hirsutum*. These are found in the bottom row of Figure 1. The phenotype for okra is missing from the D diploid series since the okra gene has never been identified in a D diploid species. Both okra and super okra may be considered to be evolutionary products of the amphidiploids.

For simplicity the extremes in phenotypic expression are reported herein. The laciniate and Asiatic "broad" alleles were transferred from the A_2 genome via new amphidiploids. The laciniate has been obtained from the A_2 of amphidiploids $2A_2B_1$ and $2A_2D_1$, and Asiatic "broad" from $2A_2D_1$. The transfer of these A_2 genes to the A genomes of the natural amphidiploids was feasible because of the high amount of chromosome homology in the A_2 and the A_h genomes in each hybrid with *G. hirsutum* $2A_hD_h$. Infertility was great in these hybrids.

The dominant gene for the "thurberi" lobation was transferred from the $2D_1$ genomes of $2A_2D_1$ to the D genomes of the natural amphidiploids. The recessive gene for entire leaf came from special hybrids. The entire-leaf genes were transferred from the D_5 and D_2 genomes to the trispecies hybrids, A_hA_2 D_hD_5 and $A_hA_2D_hD_2$, to the D_h genome of amphidiploid $2A_hD_h$. These gene transferences

were feasible because of the high degree of homology between chromosomes of the D diploid and the D_h genomes. An anomalous behavior is reported for the leaf-shape gene of $2D_1$ in its transfer to the D_h and D_b genomes.

After transference was accomplished the following hybrids were made, using leaf-shape genes of the natural amphidiploids in *G. hirsutum* and *G. barbadense* stocks. These stocks themselves often contained leaf-shape genes transferred from another amphidiploid. For example, super okra in B.(1) below was a backcross transfer from *G. hirsutum*, since super okra does not occur in *G. barbadense* (STEPHENS 1945b).

A. (1) Laciniate × super okra, *G. hirsutum* background. F_1 genotype $L^L/l\ l/L^S$
(2) Laciniate × "thurberi", *G. hirsutum* background. F_1 genotype $L^L/l\ l/L^T$

Both F_2 and backcross populations were grown. Randomly taken F_3 proof progenies were observed to confirm F_2 classification. The backcross parent was a stock of *G. hirsutum* having a broad-leaf expression and a recessive ($l\,l\ l\,l$) genotype.

B. (1) "Thurberi" × super okra, *G. barbadense* background F_1 genotype $l/l\ L^T/L^S$
(2) "Thurberi" × Sea Island, *G. barbadense* background. F_1 genotype $l/l\ L^T/L^E$
(3) "Thurberi" × okra, *G. hirsutum* background. F_1 genotype $l/l\ L^T/L^O$

Backcross populations were grown. F_2 proof progenies were observed. A broad-leaf stock was used in most cases, furnishing a recessive ($l\,l\ l\,l$) genotype. In a few instances Sea Island ($l\,l\ L^EL^E$) was used when super okra was involved.

Three methods were used to distinguish between leaf-shape phenotypes in a common population. The first two were visual approaches. The first approximation came from a climax leaf of the main stem taken 3–4 nodes above the first sympodium and 5–6 leaves below the terminal leaf. If two phenotypes appeared alike, as they often did in backcross A.(1), leaves were observed at nodes along the main stem below the sympodia. This presumed a difference in the time of initiation of leaf lobing, being a developmental pattern technique adapted from STEPHENS (1945a,b,c) and characteristic for certain diploid leaf-shape genes. The third method was to measure climax leaves and to calculate indices; each allele has a characteristic value (HUTCHINSON 1934; STEPHENS 1945b). In reference to the Sea Island leaf shape in Figure 1, the distance from the leaf pulvinus (the indention just above the "I" in Island) to the tip of the central lobe became L. The average distance from the pulvinus to the sinus of the central lobe, the point of the maximum cut in the leaf along the central lobe, was called S. The ratio S/L was taken from measurements of several leaves from several plants to determine dominance and gene interaction of alleles. The ratio W/L was obtained in a similar manner, being the width of the central lobe at its

widest place, usually at a place distal to the sinus of the leaf. The number of lobes was suggestive of particular leaf-shape genes, but this information was often inconclusive.

EXPERIMENTAL RESULTS

Genetic relationships as determined by segregation ratios

Duplicated phenotypes—duplicated genes

A deeply lobed leaf was observed in the amphidiploid $2A_2B_1$ and in its hybrids with recessive broad-leaf *G. hirsutum*. This phenotype, laciniate, was repeated in the backcross of the hybrid $A_2B_1 \times A_hD_h$ with broad-leaf stocks. A second phenotype typical of the recessive parent was present in the backcross in about the same frequency. Because of the infertility in this hybrid and in its backcross progeny, plants having laciniate in the backcross were again backcrossed with the broad-leaf stocks, and a ratio of one laciniate to one broad leaf was observed in the progeny. A third backcross repeated the one to one ratio. Laciniate plants in each backcross generation were inbred, and an F_2 segregation in a ratio of three laciniate to one broad-leaf plant was observed. The laciniate class in the F_2 populations was separated into a homozygous and a heterozygous component; separation by S/L calculated ratios was more accurate than visual classification, as judged from information from test crosses made to confirm a phenotype. The homozygous laciniate, $L^L\ L^L\ l\ l$, had S/L ratios from 0.07–0.10 and heterozygous laciniate, $L^L\ l\ l\ l$, had S/L ratios from 0.11–0.18. Similar data were obtained for laciniate from $2A_2D_1$.

A stock, now homozygous for laciniate and fully fertile, was crossed with a stock carrying super okra and the resulting hybrid (A–1) was inbred. The F_2 had 812 plants with deeply lobed leaves, S/L ratios from 0.00–0.18, and 67 plants with broad-lobed leaves, S/L ratios from 0.40–0.60. This segregation pattern has a P of 0.09 for an expected ratio of 15 dominant to one recessive. Classification in a 4:6:5:1 ratio was also made; the four had a super-okra phenotype, the six had a phenotype like that of the F_1 hybrid, and the five like heterozygous laciniate as described in the paragraph above. This 5/16 class composed one phenotypic class when mature leaves at flowering nodes were classified by measurement and visual methods; but progeny testing showed that 3/16 had been laciniate and 2/16 heterozygous super okra. The mature plant phenotype of the 5/16 class was produced by different genotypes, and a separation of genotypes was possible from juvenile plants. The first and second true leaves were much lobed in genotypes having a laciniate gene, while these leaves were essentially entire in genotypes having a super-okra gene.

The hybrid between laciniate and super okra was backcrossed to a broad-leaf stock. The duplicate factor ratio of three dominant to one recessive was observed; there were 56 deeply lobed to 23 broad-leaf plants. Both laciniate and super-okra heterozygous genotypes again produced a common phenotype. The doubly heterozygous genotype ($L^L\ l\ L^S\ l$) again produced the phenotype of the F_1 hybrid. The

largest S/L ratio value was 0.18 for any dominant phenotype, and the smallest value was 0.42 for the broad-leaf phenotype.

A homozygous laciniate stock was crossed with a stock having a lobed-leaf expression controlled by a dominant gene of $2D_1$ that had transferred ten backcross generations to *G. hirsutum*. The hybrid (A–2) was inbred and the F_2 population grown. A 15:1 ratio was expected since laciniate was an A_h genome gene and the "thurberi" a D_h genome gene. The F_2 showed 331 deeply lobed and 24 broad-leaf plants, and the backcross 76 deeply lobed to 24 broad-leaf plants. These observed values were in close agreement with the expected 15:1 F_2 and 3:1 backcross ratios. The various lobation phenotypes in the F_2 were divided into 12 "laciniate":3 thurberi:1 broad, and again each of the classes having the lobed leaves could be subdivided into homozygous and heterozygous genotypes. Interaction of leaf-shape genes changed the phenotypic expression when laciniate and thurberi genes were both present in a genotype. More about this interaction is given in the Gene action section.

Two contradictory locations of the amphidiploid leaf-shape locus were indicated earlier in the introduction. STEPHENS (1945c) suggested an A genome location; therefore laciniate being herein transferred to the A_h genome ought to be allelic with super okra. The observed ratio of 15 lobed leaf to one broad leaf, however, shows that laciniate and super okra are genes at independent loci; but this information does not place the super okra locus on the D_h genome. GREEN (1953) suggested the super okra locus was on the D_h genomes and, therefore, a 15 to one ratio is expected. He also suggested that *G. thurberi* $2D_1$ had contributed a new allele to the super okra locus, but he showed no evidence that the $2D_1$ contributed it. As expected in the hybrid laciniate and the leaf-shape gene from *G. thurberi* in a D_h genome location gave a 15 to one ratio. That "thurberi" leaf and super okra are alleles at a common D_h locus is shown below. It is germane here to note that GERSTEL and PHILLIPS (1958) reported segregation of okra and super okra-leaf shape in hexaploid hybrids $2A_hD_hD_5$. Their segregation in tetrasomic fashion involved loci of the D genomes of the hexaploid. STEPHENS (1955) subsequently reported the linkage of okra-leaf shape with crinkled of the D_h genome. The STEPHENS' hypothesis for genome location has been discarded.

Multiple allelic genes

A leaf-shape gene was transferred readily from the D_1 genome of amphidiploid $2A_2D_1$ to both *G. hirsutum* and *G. barbadense*. The Materials and Methods section indicates that interaction of the "broad" A_2 alleles and the D_1 genes produced a leaf shape similar to the Sea Island phenotype of *G. barbadense*. This expression is called "thurberi" leaf shape. In the cross of amphidiploid $2A_2D_1$ with broad leaf *G. hirsutum* the resulting hybrid plants were of two phenotypes. The thurberi leaf shape occurred in 300+ hybrid plants in a ratio of 19 to one for broad leaf. The exceptional broad-leaf hybrids also had all of the dominant genes of the A_2 genome that the thurberi-leaf hybrids had. In a cross of amphidiploid $2A_2D_1$ with *G. hirsutum* where the laciniate gene was used on the A_2 genome, GERSTEL and PHILLIPS (1957) indicated one plant only that did not have the laciniate ex-

pression; however, they considered the segregation between A and D genomes to be infinity to zero (no segregation). These two independent cases should be considered to be intergenomic A_2–D_1 segregation. BEASLEY (1940, 1942) reported occasional quadrivalents in his $2A_2D_1$ hybrid; genetic segregation would be expected from the particular A–D chromosomes involved in the anomalous quadrivalents.

Both the thurberi and the broad-leaf hybrids were crossed reciprocally with broad-leaf stocks of *G. hirsutum* The thurberi phenotypes segregated in a typical ratio of one thurberi to one broad-leaf pattern. One exceptional plant with an extra narrow leaf produced a good-fitting three thurberi to one broad ratio and was L^T L^T *ll*. The broad-leaf hybrids did not segregate nor did any of the randomly drawn broad-leaf plants of the test cross population. An example of typical segregation from a thurberi hybrid is a 1953 backcross population which was grown under summer (Arizona) conditions. There were 81 thurberi and 87 broad-leaf plants. Additional backcrossing repeated the one to one segregation ratio. The thurberi phenotype changed but little as the gene from D_1 was transferred to *G. hirsutum* D_h genomes by backcrossing.

Hybrids were made between the original $2A_2D_1$ amphidiploid and super okra and okra stocks; these crosses (B1,B3) are listed in the Materials and Methods section. The resulting hybrids were backcrossed with recessive stocks. The (B–1) hybrid having super okra and backcrossed to L^E L^E *ll* produced 64 plants with a super okra heterozygous phenotype and 47 with thurberi and/or Sea Island, plus five broad-leaf plants. Randomly drawn plants from each phenotype were again backcrossed; the super okra heterozygotes produced only super okra and Sea Island plants in a typical 1:1 ratio. The thurberi or Sea Island phenotypes rarely segregated. The broad-leaf plants produced some but not all broad-leaf plants. The (B–3) hybrid having okra produced 306 deeply lobed and seven broad-leaf plants, and 127 of the 306 plants were classified as heterozygous okra and 179 as heterozygous thurberi. The phenotypes overlapped considerably in this interspecific backcross population. However, randomly drawn plants when testcrossed produced a one to one segregation from okra phenotypes and one to one from thurberi phenotypes. There was no error of classification. Subsequently after several backcrosses to broad-leaf stocks, plants having thurberi phenotypes were crossed with okra stocks, i.e., L^T/L^O hybrids were produced, and the F_2 segregation showed a good fit to a ratio of one thurberi; two F_1 phenotypes; one okra, and zero broad-leaf plants. Undoubtedly the thurberi leaf-shape gene was an allele of super okra and okra at a D_h genome location.

The occurrence of broad-leaf plants from the (B–1,B–3) hybrids should be considered to be intergenomic (A–D) segregation. MENZEL and BROWN (1954) diagrammed associations of chromosomes which indicated A-D pairing in hybrids similar to these. The replacement of a D_h or D_1 leaf-shape gene by the gene for broad leaf of A_2 would be detected as plants lacking a dominant leaf-shape gene, and 12 plants from (B-1, B-3) hybrids did not exhibit a dominant gene. Additional chromosomes besides those having leaf-shape genes could have been involved in

the A-D pairing as MENZEL and BROWN (1954) illustrated cytologically. Plants lacking allelic dominant genes for pollen color, corolla color, and petal spot of the A_2 and A_b or A_h genomes were detected. Dominant alleles of the D_1 and D_h genomes other than the leaf-shape genes were missing in the first backcross generation.

Genetic relationships as determined by gene action

Identical phenotypes = Controlled by either identical genes or similar alleles

Genetic tests showed no segregation for a number of genes of diploid species transferred to amphidiploid genomes. The data on gene action, as indicated by phenotype expression, dominance to known alleles, and interaction with leaf-shape genes at independent loci, were interpreted thusly: (a) The diploid gene was so similar to the amphidiploid leaf-shape gene in action that it was equivalent and probably identical with the amphidiploid gene at the particular locus, and (b) the diploid gene was similar to but not necessarily identical with an allele at a particular locus. Certain genes closely linked with the leaf-shape locus made identification of alleles at a locus uncertain. This stage (b) was reached by STEPHENS (1945a,b) when additional backcrossing did not refine the phenotypic expression of suspected "identical" alleles. The (b) stage also was reached in this study. Closely linked genes of known linkage groups from diploid species were shown to remain in parental combinations after many backcross generations (RHYNE 1958). In the absence of any means for breaking such close linkages, other than irradiation which can cause rearrangement, no effort is made here to separate the (a) and (b) patterns.

The gene for Asiatic "broad", the typical allele of *G. herbaceum* $2A_1$, was available herein in the $2A_2$ genome. When transferred to the A_h and A_b genomes, it did not segregate distinctly from the genes for the recessive broad-leaf phenotype. These A genome genes can be considered to be equivalents in gene action.

The gene for entire leaf, the recessive alleles of either *G. raimondii* $2D_5$ or *G. armourianum* $2D_2$, is equivalent to the allele at the D_h genome locus that is recessive to okra leaf shape.

The interaction of recessive Asiatic "broad" and recessive entire in amphidiploid background produces a broad-leaf phenotype. This result is in agreement with STEPHENS' hypothesis (1945c) that an interaction of recessive leaf-shape genes of the Asiatic A genomes with genes for entire leaf shape of $2D_5$ produces the broad-leaf expression of the amphidiploid species.

In the (B–1,2) hybrids the gene for thurberi leaf-shape did not segregate distinctly from the gene for typical Sea Island expression in *G. barbadense* background. A distinction could be made in late-flowering plants, where a thurberi expression was associated with late flowering and Sea Island with early flowering. Additional backcrossing failed to show that the leaf shape of the late-flowering plants was consistently thurberi; rather it appeared that in late-flowering background a Sea Island genotype would be modified into a thurberi expression. Attempts to free the leaf lobation from flowering influence were unsuccessful.

In *G. hirsutum* the thurberi expression was characteristic with a tendency for thurberi lobation to occur in late-flowering phenotypes. (It is worth while to remark that a Sea Island expression in *G. hirsutum* results from an introgressed gene of *G. barbadense*. Certain of the associated characters accompanying the *G. barbadense* gene are not removed by backcrossing.) The thurberi and Sea Island genes are of the (b) pattern.

Duplicated phenotypes—not necessarily determined by duplicated genes

Certain phenotypes of known leaf-shape genes of *G. hirsutum* were produced by various combinations of the laciniate gene from the A_2 genome in an A_h genome location. One level of laciniate, heterozygous at the A_h locus in an $L^L\ l\ l\ l$ genotype, produced a phenotype equivalent to that of heterozygous super okra, an $l\ l\ L^S\ l$ genotype. Two laciniate genes, homozygous in the A_h genomes in an $L^L\ L^L\ l\ l$ genotype, produced a phenotype equivalent to homozygous okra, an $l\ l\ L^o\ L^o$ genotype. (This was as STEPHENS 1945c predicted.) Two laciniate genes and two okra genes in an $L^L\ L^L\ L^o\ L^o$ genotype produced a phenotype equivalent to homozygous super okra, an $l\ l\ L^S\ L^S$ genotype. The potency of the L^S genes suggests that changes have occurred at the leaf-shape locus in the evolution of the amphidiploids.

Another way of showing similarity of phenotypes may be taken from data reported in Tables 1–3. In Table 1 the S/L ratios for two varieties of *G. hirsutum* are shown; e.g., the S/L ratio in one variety for heterozygous laciniate is 0.130.

TABLE 1

Phenotypic expression of laciniate genes in the A_h genome interacting with homozygous recessive genes of the D_h genome locus and dominance relationships of laciniate to broad

Genotype	Phenotypic expression observed	Dominance information			
		Expected value $(G_1+G_3)/2$	Deviation (expected minus observed)	Change $(G_1-G_3)/2$	Dominance value of L^L $d=$(dev./change)
		S/L ratio values			
$G_1 = l\ l\ l\ l$	0.545a*				
(Broad)	0.447b				
$G_2 = L^L l\ l\ l$	0.172a	0.320a	0.148a	0.224a	0.66a
	0.130b	0.268b	0.138b	0.179b	0.77b
$G_3 = L^L L^L l\ l$	0.096a				
	0.089b				
		W/L ratio values			
$G_1 = l\ l\ l\ l$	0.559a				
	0.552b				
$G_2 = L^L l\ l\ l$	0.294a	0.386a	0.092a	0.172a	0.53a
	0.308b	0.424b	0.116b	0.128b	0.90b
$G_3 = L^L L^L l\ l$	0.214a				
	0.297b				

* The a and b represent different varietal backgrounds of *G. hirsutum* in different growing seasons.
** For explanation of S/L and W/L ratios see last section under Materials and Methods.

TABLE 2

Phenotypic expression of three alleles of the D_h genome interacting with homozygous recessive genes of the A_h genome locus and dominance relationships to broad

Genotype	Phenotypic expression observed	Dominance information			
		Expected value $(G_1+G_5)/2$	Deviation (expected minus observed)	Change $(G_1-G_5)/2$	Dominance value of alleles $d=$ (dev./change)
		S/L ratio values			
$G_1 = l\ l\ l\ l$ (Broad)	0.545a*				
	0.447b				
	0.517c				
$G_4 = l\ l\ L^s\ l$	0.132a	0.279a	0.147a	0.266a	0.55a (super okra)
$l\ l\ L^t\ l$	0.261b	0.298b	0.037b	0.149b	0.25b (thurberi)
$l\ l\ L^o\ l$	0.184c	0.298c	0.114c	0.219c	0.52c (okra)
$G^5 = l\ l\ L^s\ L^s$	0.013a**				
$l\ l\ L^t\ L^t$	0.150b				
$l\ l\ L^o\ L^o$	0.079c				
		W/L ratio values			
$G_1 = l\ l\ l\ l$ (Broad)	0.559a				
	0.552b				
	0.541c				
$G_4 = l\ l\ L^s\ l$	0.293a	0.342a	0.049a	0.216a	0.22a (super okra)
$l\ l\ L^t\ l$	0.465b	0.492b	0.027b	0.060b	0.45b (thurberi)
$l\ l\ L^o\ l$	0.340c	0.363c	0.023c	0.178c	0.13c (okra)
$G_5 = l\ l\ L^s\ L^s$	0.126a				
$l\ l\ L^t\ L^t$	0.432b				
$l\ l\ L^o\ L^o$	0.155c				

* The a, b and c represent different varietal backgrounds of *G. hirsutum* in the same growing season.
** This value ordinarily has a 0.000 value, since there is a single lobe. Occasional leaves on an occasional plant have a small sinus of about 0.02, hence average was 0.013.

In Table 2 the S/L ratio for heterozygous super okra is 0.132 for the same varietal background. The W/L values of the two heterozygous genotypes is the same, about 0.290. Similarly homozygous laciniate had a value of 0.089 for the S/L ratio in Table 1 and homozygous okra had a value of 0.079 in Table 2. The two varieties are not the same, but the flowering responses are comparable. In each of these duplicated phenotypes the number of leaf lobes also was the same. There was no doubt that a common phenotype could be produced by genes of different genomes.

A pertinent question is whether a common phenotype is produced by the same gene when present in either one of the genomes of an amphidiploid. The question perhaps is moot since linkages of leaf-shape genes and adjacent loci have resisted recombination in backcross transference procedures. Even so certain evidence that a common phenotype is produced by different genes in each genome can be marshalled. This evidence depends on estimates of dominance and epistatic gene interaction. In Tables 1–3 dominance is taken to be the ratio (d), the deviation

TABLE 3

Phenotypes of two alleles of the A_h genome loci interacting with three alleles of the D_h genome loci

Alleles of the A_h genome	Phenotypes expressed as ratios: Alleles of the D_h genome locus					
	$L^S L^S$	$L^t L^t$	$L^S l$	$L^t l$	$l\ l$	$l\ l$
	S/L ratio values					
$l\ l$	0.013	0.150	0.132	0.261	0.545	0.447
	G_5a	G_5b	G_4a	G_4b	G_1a	G_1b
$L^L l$	0.002	0.056	0.049	0.142	0.172	0.130
	G_7a	G_7b	G_6a	G_6b	G_2a	G_2b
$L^L L^L$	0.000	0.049	0.069	0.080	0.096	0.089
	G_9a	G_9b	G_8a	G_8b	G_3a	G_3b
	W/L ratio values					
$l\ l$	0.126	0.432	0.293	0.465	0.559	0.552
	G_5a	G_5b	G_4a	G_4b	G_1a	G_1b
$L^L l$	0.100	0.267	0.142	0.310	0.294	0.308
	G_7a	G_7b	G_6a	G_6b	G_2a	G_2b
$L^L L^L$	0.090	0.181	0.195	0.303	0.214	0.297
	G_9a	G_9b	G_8a	G_8b	G_3a	G_3b

of the heterozygous genotype from the expected midpoint divided by the expected change in phenotype between the two homozygous genotypes. In Table 1, for example, the expected midpoint or G_2 (heterozygous laciniate) is ½ of $G_1 + G_3 = 0.320$. The observed G_2 value for the S/L ratio (variety background a) is 0.172. The observed midpoint deviates from the expected, or $0.320 - 0.172 = 0.148$ is the deviation. The dominance of laciniate makes the phenotype of G_2 more deeply cut than expected. The expected change, 0.224, in phenotype due to one laciniate gene in G_2 is ½ of $G_1 - G_3 = 0.224$ (the actual change resulting when $2L^L$ genes were substituted for l in the A_h genome). The dominance value of L^L is $d =$ deviation divided by change or $0.148/0.224 = 0.66$ for variety a. The complete dominance of laciniate to broad would have produced an actual change of 0.224 and a ratio of $0.224/0.224 = 1.00$. The heterozygous G_2 and homozygous G_3 genotypes also would have had the same phenotype and same measurement.

In Table 1 the dominance relations (d) of the laciniate gene are given for common varietal backgrounds, where laciniate is interacting with the genes for entire leaf of the D_h genomes. The laciniate gene is highly dominant for the S/L ratio in the heterozygous condition, as is indicated by the d value of about 0.70. In Table 2 the dominance relations of the super okra and okra genes interacting with A_h broad leaves are given. A value of about 0.50 fits the two D_h alleles. By assuming that the recessive genes of the A_h and the D_h are the same, i.e., the broad leaf of Asiatics and the entire leaf of D diploids are merely the expressions (STEPHENS 1945c) manifest in the two diploid backgrounds, and by further as-

suming that a dominant gene would produce the same lobing in a amphidiploid regardless of which genome carries the gene, the difference in the levels of dominance suggests that laciniate and okra are not the same genes. A more plausible speculation is that the recessive genes of the A_h are not the same as the recessive genes of the D_h genomes, therefore a difference in the level of dominance between the laciniate and the okra genes is expected.

The data of Table 2 suggest that thurberi leaf is not a true allele of the super okra series of genes. Its dominance is much less than that of either super okra or okra as calculated from the S/L ratio. In parental $2D_1$ (Figure 1) the leaf has a small sinus and an S/L ratio of 0.01–0.02. This ratio compares with 0.110–0.120 for laciniate of the *G. arboreum*. A transference of thurberi to the D_h genome produces a value of 0.150–0.180 for the S/L ratio (Table 2), while laciniate in the A_h genome shows 0.080–0.100 (Table 1). Furthermore, thurberi interacts with super okra and okra in heterozygotes: the S/L ratio is 0.00 for $L^T L^S$ and essentially 0.15 (near the thurberi value) in L^T/L^O heterozygotes. This suggests that okra is not dominant to thurberi but is to entire.

The information of Table 3 for two populations conforms to different quantitative inheritance models. The phenotypes of the nine genotypes expected in an F_2 do not support the duplicate-gene model. The genotypes having a super-okra gene and a laciniate gene produce a number of variations in phenotypes, not the single phenotype as is expected from duplicated dominant genes. There was interaction of genes in a manner somewhat similar to a model for an additive gene action in quantitative inheritance studies. The doubly heterozygous genotype $L^L\, l\, L^T\, l$ produces a phenotype that deviated from what might have been predicted on the basis of the homozygous parental genotypes. Variations like the latter, an epistatic model, were encountered when genes for lobed leaf (other than laciniate) from the A_2 genome are combined with the various D_h alleles in F_2 populations.

DISCUSSION

The leaf-shape locus of the natural amphidiploid species is placed on the D genomes (1) by the evidence that the gene for thurberi leaf, by transference, at a D_h locus is an allele of the super-okra series of genes and (2) by obtaining a duplicate factor ratio when the laciniate gene on the A_h genome is brought into an F_2 population with either the thurberi or the super-okra genes. This placement of the super-okra locus agrees with independently published evidence for a D genome location in the amphidiploid species.

In a new amphidiploid, as stated previously, the leaf-shape genes of the parental diploid genomes should be the same as leaf-shape genes of the amphidiploids. This expectation was confirmed for certain genes only of the A and D diploid genomes: (1) The genes which interact in amphidiploid background to produce a broad-leaf shape are the recessive genes for broad leaf of the A diploid genomes and the recessive genes for entire leaf of the D diploid genomes. (2) The genes which interact in amphidiploid background to give Sea Island expression are

equivalent to the recessive genes for broad leaf either of the diploid or amphidiploid A genomes and the gene for thurberi leaf of the $2D_1$ diploid species. Another way of producing the same phenotype also has been observed. A gene from the A_2 genome, the L^r allele, has been transferred to the A_h genome, producing a thurberi and/or Sea Island phenotype. Its genome location is its chief distinction from the Sea Island phenotype. STEPHENS (1945c) also suggested that lobing gene, L^N of A_2 would interact with an entire leaf and give the Sea Island expression. Even so, in the settling down of a new amphidiploid a transfer of genetic material from one genome to another is entirely possible. A transfer of thurberi to the A and recessive broad to the D genomes was shown for amphidiploid $2A_2D_1$ and its hybrids with *G. hirsutum* and *G. barbadense*. Sea Island might have been an A genome gene, but in the D genome it is stable as STEPHENS (1945c) reported for *G. barbadense*. It is not commonly found in *G. hirsutum*. It usually is found in types that have been introgressed with *G. barbadense*.

A number of phenotypes similar to normally occurring leaf shapes were produced by leaf-shape genes from the diploid genomes when transferred into amphidiploid background. Genes at independent loci were indicated by the F_2 segregation ratios, and the gene action studies suggested that the same gene probably was not duplicated at each locus. The laciniate and super-okra genes, each on a different genome, interacted and produced phenotypic expressions in F_2 that were typical of additive gene action in quantitative inheritance models. The laciniate and thurberi genes, also on different genomes, produced phenotypic expressions in F_2 typical of epistatic gene action in quantitative inheritance models. A duplication of a phenotype thus does not require the same genes or even identical gene actions. These diversifications sharply contrast with the duplicate dominant gene model often expected for an amphidiploid. The ability to produce a common phenotype by different gene actions is suggestive rather of an evolving amphidiploid; that is to say, diploidization is occurring in the amphidiploids. The gene action could suggest that the Gossypium amphidiploids are probably not recent in origin, and perhaps not even of a common origin.

Mutations of broad-leaf to okra and okra to superokra are recorded in the literature (STEPHENS 1945c). These three are the alleles of *G. hirsutum*, either from collections made in the wild or from cultivated forms. The *G. barbadense* collections show essentially but one allele, Sea Island, except for a rare occurrence of okra in var. *darwinii* STEPHENS (1945c). The equivalence of thurberi and Sea Island in *G. barbadense* background, the dissimilarity of gene action of *thurberi* (and presumably Sea Island) and the super okra and okra alleles, and the reduction of recombination in *G. hirsutum* linkages transferred to *G. barbadense*,[2] therefore collectively indicate divergence of *G. hirsutum* and *G. barbadense*. Alternatively these independently collected data may support a contention that the D genomes of *G. hirsutum* and *G. barbadense* could differ because each had

[2] Unpublished data of the writer, and DR. S. G. STEPHENS, Genetics Dept., N.C. State College, transferring. *G. hirsutum* to *G. barbadense* and *G. barbadense* linkages to *G. hirsutum*, respectively.

a different D diploid parent. (It is not possible at present to distinguish the two possibilities.) Evidence in support of divergence of D diploid and D amphidiploid genomes may be inferred from GERSTEL and PHILLIPS' 1958 report that the D diploid genomes, including the D_5 putative diploid parent of the D genomes HUTCHINSON *et al.* (1947), show less segregation than A diploid genomes in hexaploid hybrids involving *G. hirsutum* or *G. barbadense* and these diploid genomes.

SUMMARY

A transference of leaf-shape genes was made from the A and D Gossypium diploid species to the A and D genomes of natural Gossypium amphidiploids. The dominant genes from the A diploids gave duplicate factor inheritance with the dominant alleles of the amphidiploid occurring at the leaf-shape locus. The dominant genes of the D diploids exhibited allelism with the four alleles of the amphidiploid leaf-shape locus. The independence of the A diploid genes and the allelism of the D diploid genes collectively support a placement of the amphidiploid leaf-shape locus on the D genomes. The gene from *G. thurberi* $2D_1$ was commonly transferred to the D genomes of amphidiploids and infrequently to the A genomes. The transposition of this D diploid gene to each of the amphidiploid genomes perhaps illustrates a similar earlier transposition of an A genome gene during the settling down of a newly produced amphidiploid. The "thurberi" gene for leaf shape was shown to be similar to the Sea Island gene commonly found in *G. barbadense*. The entire leaf-shape gene from two different D diploids was similar and perhaps identical with the gene now present in the D genomes of broad-leaf *G. hirsutum* $2(A_hD_h)$.

A production of a common phenotype was possible from more than one combination of genes of the A and D diploids in amphidiploid background. Such diversification in gene action by the genes at each of the two amphidiploid loci was unexpected for the Gossypium amphidiploids, which sometimes have been assumed to be of recent and common origin. Rather this ability to produce a common phenotype with different genes of each genome of an amphidiploid suggests that Gossypium amphidiploids are evolutionary old species. Alternatively, the amphidiploids may not be old species but may have multiple origins at least of the D genomes. The gene interaction of the leaf-shape genes of each locus of amphidiploid *G. hirsutum* produced phenotypes that resembled models for epistatic gene action in quantitative inheritance studies. Neither the duplicate model nor the additive gene action model had a good fit to the observed interaction of leaf-shape genes in the amphidiploid.

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