THE origin, fertility, and identifying vegetative characteristics associated with some monosomics of Upland cotton, *Gossypium hirsutum* L., have been described by Brown and Endrizzi (1963). The monosomes were highly stable and n–1 gametes were transmitted with a high frequency through the egg, but very rarely through the pollen. Endrizzi and Brown (1963) have obtained the cytogenetic data that identified monosomes for six of the 26 chromosomes of cotton. The six monosomics differ from each other and from normal disomics in specific syndromes of morphological features which are sufficiently distinct in a wide range of genetic backgrounds to permit accurate separation of monosomic from normal types.

*G. hirsutum* is an amphidiploid, and consequently its genetic analysis for quantitative characters is somewhat difficult and less conclusive as compared to similar analyses in diploid species. To a great extent this difficulty is caused by the duplication of quantitative and qualitative factors located on several chromosomes which can interact additively and nonadditively and greatly influence the phenotypic expression of a trait. Since each variety or breeding stock may possess a different complement of alleles, segregating hybrid generations present difficulties in measuring and analyzing the genetic components determining a particular character.

The establishment of the monosomics of cotton in a uniform genetic background provides a means of replacing any one chromosome by its homologue of another variety or breeding stock and evaluating it genetically. The substituted chromosome would be present intact in essentially a uniform genetic background; such substituted lines can be increased and performance tested in replicated field trials with the recipient line and the donor line. Furthermore, as applied to cotton breeding and the manipulation of genes that condition plant characters of economic value, the monosomic testing technique just described makes it possible to associate the effects of a gene (or group of genes) with a definitely identified or "marked" chromosome. Such studies are now in progress at this station.

In addition to their value in associating genes affecting agronomic characters

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on specific chromosomes, monosomics may be used for associating simple Mendelian factors with specific chromosomes. Only small populations are required for assigning gene loci to the single chromosome of monosomics.

In this report six primary monosomes and one tertiary monosome involving a translocation (to be described later) were used for testing the association of ten dominant mutant genes with specific chromosomes.

MATERIALS AND METHODS

The amphidiploid cotton, *G. hirsutum*, has the genome formula 2AD. Four of the six primary monosomes have been identified as chromosomes 1, 2, 4 and 6 of the A subgenome, and the remaining two have been identified as chromosomes 17 and 18 which belong to the D subgenome of *G. hirsutum* (Endrizzi and Brown 1963).

Some of the monosomes were recovered in lines homozygous or heterozygous for one or more marker loci; others were recovered in lines having only standard or normal alleles. The monosomic plants that carried marker genes at the time of recovery were used directly in tests for association of the marker gene and the monosome. Monosomics having the normal or standard alleles were crossed to stocks carrying marker genes, followed by testing the monosomic F1. In earlier tests the monosomic F1 was used as female only in the backcross; in later tests, however, the monosomic F1 was crossed reciprocally with the tester stocks.

The tertiary monosomic involving the translocation (Z2229) showed metaphase I pairing of 24 bivalents and a chain of three chromosomes instead of 25 bivalents plus a univalent as in primary monosomes.

According to the system proposed by Menzel and Brown (1954) for numbering the ends of the first five A subgenome chromosomes of *G. hirsutum*, chromosome 2 has ends 3-5 and chromosome 3 has ends 4-6. The chromosomes and their end arrangements in the chain of three were identified by Endrizzi and Brown (1962) as being 3-5-6-4 (Figure 1) with the change in homology being close to the centromeres. They proposed that the deficiencies were the result of unequal disjunction of an interchange complex. As shown in Figure 1, *G. hirsutum* plants with the chain are deficient for arm 3 of chromosome 2 and arm 4 of chromosome 3 (the extent of the deficiency for either arm will, of course, depend on the position of the break).

![Figure 1](image_url)

**Figure 1.**—Diagrammatic representation of the interchange chain of three chromosomes which is deficient for “arm” 3 of chromosome 2 (3-5) and “arm” 4 of chromosome 3 (4-6). C signifies probable region of the centromere.
Chromosome 2 (3-5) has been obtained as a monosome (Table 1), but not chromosome 3 (4-6). However, since chromosome 3 is in the chain but haplo for arm 4, the association of gene loci with arm 4 of chromosome 3 can be determined. Moreover, a test for association with the entire length of chromosome 3 while in the chain can be made with any linkage group in which the terminal loci are in opposite arms, especially when they overlap the position of change in homology.

The marker stocks used in crossing with the monosomics, or that had contributed marker loci to monosomic plants at the initial time of isolation of the monosomic, and the alleles they carry are as follows:

Texas 429 (SL7-9): R₁-red plant body; \( L^o \)-deeply palmately lobed "Okra" leaf; \( R^p \)-petal spot; \( Lc^p \)-brown lint color; \( N \)-naked or slick seed. \( R^p, Lc^p, N \) are linked loci in a chromosome of the A subgenome (Harland 1935; Stephens 1955). \( L^o \) is in a D subgenome chromosome (Green 1953) and linked with the locus for green lint (Stephens 1955), a marker in Texas 562.

Texas 586: Includes the same markers as in Texas 429 plus \( Y^p \)-yellow petal; \( Pa \)-yellow pollen; \( H^p \)-Hairy (pilose) plant body. These three markers have been placed in the A subgenome (Stephens 1954a,b; Gerstel and Phillips 1958).

Texas 562 (SM2): \( Y^p, Pa, H^p, Lg \)-green lint color.

Brymer brown lint: \( Lc^p \); \( H^p \) and \( Lc^p \) are linked (Stephens 1955).

D18: \( Pa \).

All of the above marker genes are dominant to alleles in commercial varieties of Upland cotton. Recessive tester stocks used were commercial varieties or inbred lines of Upland cotton, and in nearly all cases the tests were made with the inbred lines Deltapine 14 and 15.

Monosomics have been used frequently for genetic analysis in wheat and tobacco, and several investigators in these two crop genera have described the methods employed (Claussen and Cameron 1944; Unrau 1950; Sears 1953; Kuspira and Unrau 1959). Therefore, only the general outline of the procedures employed in the present study will be given. These procedures are applicable to either whole or part chromosome deficiencies. In the procedures which are outlined below, dominant and recessive refer to phenotypes of individual plants.

\( P_1 \) haplo-X recessive females \( \times P_2 \) diplo-X dominant

\( F_1 \) haplo-X dominant + diplo-X dominant

(a) Expected results if marker and haplo-X are not associated:
1. \( F_1 \) haplo-X dominant females \( \times \) diplo-X recessive
   Backcross haplo-X dominant and recessive + diplo-X dominant and recessive
2. Reciprocal cross (only n pollen is functional)
   diplo-X recessive females \( \times F_1 \) haplo-X dominant
   Backcross diplo-X dominant and recessive
3. \( F_1 \) haplo-X dominant selfed
   \( F_2 \) haplo-X dominant and recessive + diplo-X dominant and recessive

(b) Expected results if marker and haplo-X are associated:
1. \( F_1 \) haplo-X dominant females \( \times \) diplo-X recessive
   Backcross haplo-X recessive + diplo-X dominant
2. diplo-X recessive females \( \times F_1 \) haplo-X dominant
   Backcross diplo-X dominant
3. \( F_1 \) haplo-X dominant selfed
   \( F_2 \) haplo-X dominant + diplo-X dominant
Under (a) above, in which the $F_1$ haplo-X dominant is used as female in the testcross, both dominants and recessives are present in each of the cytological classes. When the $F_1$ haplo-X dominant is used as the male in the backcross, all individuals are disomic, since only $n$ pollen grains function; here again dominants and recessives are present. Self pollinating the haplo-X $F_1$ produces an $F_2$ population in which both dominants and recessives are present in each cytological class. Hence, there is no association of monosome and marker in any of the three procedures.

Under (b) in which haplo-X $F_1$ is used as female in the backcross, the two phenotypic and cytological classes are also present, but here all haplo-X plants are recessive and all diplo-X plants are dominant. In the reciprocal backcross all individuals are disomic and of only one phenotype—the dominant. Self pollinating the haplo-X $F_1$ results in an $F_2$ population with the dominant phenotype only. It is clearly evident in each of the three cases that the monosome and the marker locus are associated.

The marker genes employed are dominant to alleles in commercial varieties of Upland cotton; therefore, in the interpretation of the results only two basic assumptions need be made: (1) for dominant mutants the dominant phenotype will be expressed if the dominant mutant gene is present not only in the homozygous and heterozygous but also in the hemizygous condition; and (2) the recessive (normal) phenotype will be expressed if the recessive gene is present not only in the homozygous but also in the hemizygous condition.

The tests reported here were conducted over a period of several years. In earlier tests cytological analyses were completed on almost all progenies to insure correct identification of monosomic and disomic individuals. This thorough testing proved that monosomic plants were consistently different from normal plants in certain plant characters and that these diagnostic characters can be used to separate the two cytotypes. In recent tests, therefore, individuals in the two cytological classes were identified only by their phenotypically diagnostic characters.

For markers that are in known linkage groups, it would have been sufficient to test only one of the markers in each group against each primary monosome to determine whether the linkage group is on a specific chromosome; furthermore, for those markers which have been assigned to one of the subgenomes by genetic tests, one might consider it redundant to check them against chromosomes not belonging to that subgenome. However, since the current series of tests for association of monosomes and markers are the first to be conducted in cotton, all markers were scored for the additional information in determining the feasibility of the monosomic technique as a means of assigning genes to specific chromosomes of cotton.

The number of progenies scored varied among some of the tested combinations (Table 1). This was due to the testing of monosomic plants that already had at least one of the marker genes in their genotype, the duplication of some marker loci among the marker stocks, and the scoring for segregation of one or more markers in monosomes of different origin which later proved to be duplicates.
RESULTS AND DISCUSSION

**Primary monosomes.** The results of 73 tests involving the six primary monosomes and ten marker genes are presented in Table 1. The segregation ratios in Table 1 show independence of the marker and the monosome in all combinations tested except \(H_{s}\) and \(Lc_{s}\) with haplo-6. Independence is shown by individuals of the two phenotypic classes appearing as disomics and monosomes in the backcrosses for all monosomes and in the \(F_{2}\) populations for one monosome. This is the pattern of segregation expected when a monosome and a marker are not associated.

The exceptional combination is the association of \(H_{s}\) (pilose) and \(Lc_{s}\) (brown lint) with haplo-6. The data in Table 1 show that when the \(F_{1}\) haplo-6 pilose was crossed as female to diplo-nonpilose, only two of the four expected classes were recovered, the diplo-pilose and the haplo-nonpilose. Furthermore, when the \(F_{1}\) haplo-6 pilose was crossed as male to diplo-nonpilose, only plants with the pilose phenotype were recovered. These results are expected if a monosome and a marker are associated. \(H_{s}\) and \(Lc_{s}\) are linked loci, and therefore, the association of \(H_{s}\) with chromosome 6 automatically places \(Lc_{s}\) in that chromosome. However, the Brymer brown lint line was tested with haplo-6 to verify that this line carries \(Lc_{s}\) and not one of the three other loci determining brown lint in the tetraploid cottons. Table 1 shows that the cross of \(F_{1}\) haplo-6 brown lint as female to diplo-white lint gave only diplo-brown lint and haplo-white lint, and that self pollinating haplo-brown lint gave an \(F_{2}\) population, all of which had brown lint.

Haplo-6 was shown by Endrizzi and Brown (1963) to belong to the A subgenome; therefore, the association of \(H_{s}\) \(Lc_{s}\) with haplo-6 places this linkage group without doubt in the A subgenome. The placement of \(H_{s}\) \(Lc_{s}\) on chromosome 6 and the further positioning of \(H_{s}\) in the proximal region of the long arm of this chromosome (Endrizzi 1962) represents the first case in cotton of the association of marker loci with a specific chromosome.

In genetic tests for linkage in cotton, Stephens (1955) reported that \(H_{s}\) and \(Lc_{s}\) were inherited independently of all other markers listed in Table 1. The present study not only confirms this, but moreover, since the conventional method of mapping chromosomes does not reveal linkage between genes which are so far apart as to show 50 percent recombination, the present data firmly established that \(H_{s}\) and \(Lc_{s}\) are on a chromosome which is independent of all marker loci used in this study.

**Chain of three chromosomes (tertiary monosome).** Figure 1 illustrates the general structural relationship of the two normal and the one interchange chromosomes composing the chain. Stephens (1955) has shown that the genetic map of linkage group I is \(R_{s} 52 Lc_{s} 44.4 N_{s}\), with the centromere near the \(Lc_{s}\) locus. This evidence places the terminal loci well out in opposite arms of the chromosome.

On the basis of the above observations it may be assumed that if \(R_{s}\) \(Lc_{s}\) \(N_{s}\) linkage group is on chromosome 3 (ends 4–6 in Figure 1) of the chain configuration,
### TABLE 1

**Ratios in backcross and F<sub>2</sub> populations from monosomic F<sub>1</sub>'s**

<table>
<thead>
<tr>
<th>Cross</th>
<th>Cytotype of progeny</th>
<th>Linkage group I</th>
<th>Marker genes</th>
<th>Linkage group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R&lt;sub&gt;1&lt;/sub&gt;</td>
<td>r&lt;sub&gt;1&lt;/sub&gt;</td>
<td>L&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>Monosomes of A subgenome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; haplo-1 × tester</td>
<td>2n</td>
<td>3</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2n-1</td>
<td>4</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; haplo-2 × tester</td>
<td>2n</td>
<td>13</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2n-1</td>
<td>12</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; haplo-4 × tester</td>
<td>2n</td>
<td>5</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2n-1</td>
<td>4</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; haplo-5 × tester</td>
<td>2n</td>
<td>20</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>2n-1</td>
<td>14</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Tester × F&lt;sub&gt;1&lt;/sub&gt; haplo-6</td>
<td>2n</td>
<td>29</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; haplo-6 selfed</td>
<td>2n</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>2n-1</td>
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<tr>
<td>Monosomes of D subgenome</td>
<td></td>
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<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; haplo-17 × tester</td>
<td>2n</td>
<td>5</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2n-1</td>
<td>6</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Tester × F&lt;sub&gt;1&lt;/sub&gt; haplo-17</td>
<td>2n</td>
<td>11</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; haplo-18 × tester</td>
<td>2n</td>
<td>6</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2n-1</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt; haplo-18 selfed</td>
<td>2n</td>
<td>6</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2n-1</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
the terminal loci would most likely overlap the position of change in homology, thus placing one of the terminal loci, \( R \) or \( N \), in haplo-arm 4, as illustrated in Figure 1. Even if the breakage point was such that the linkage group failed to overlap the position of change in homology, one of the loci would show essentially complete linkage with the point of change in homology, since the chain of three has never been observed with an interstitial chiasma. If the assumptions are correct it is possible then to test for association of chromosome 3 with the linkage group. The chain of three is also haplo for arm 3 of chromosome 2, but this chromosome has been obtained as a monosome and found to be independent of \( R, Lc, N \) (Table 1).

A plant with the chain of three chromosomes was crossed with Texas 586 which carries linkage group I. The two unlinked A subgenome markers \( Y \), and \( Pa \) are also in Texas 586, and therefore, the segregation of these two factors was recorded in the backcross populations; however, these two loci could be checked only for their presence or absence in the haplo-segments.

In the \( F_1 \) generation of the chain of three chromosomes \( \times \) Texas 586, two cytological types were recovered, one with 26 bivalents and the other with 24 bivalents and a chain of three. The two types could be distinguished morphologically. The \( F_1 \) plants used in the backcrosses were analyzed cytologically to confirm their cytotype, but in the backcross population, the diagnostic morphological characters were used to identify plants with one or the other cytotype. Plants with 24 bivalents and the chain of three chromosomes are shorter and have distinctly smaller leaves than sibs having 26 bivalents. An \( F_1 \) sib with 26 bivalents was also backcrossed to the recessive to serve as a control.

Since deficiencies for segments of two chromosomes are involved in the chain of three, the test for linkage of a locus and the haplo-segments is the same as that outlined for monosomic linkage analysis, i.e., observing for complete absence of an allele in certain classes depending on whether the \( F_1 \) is used as male or female in the backcross. As is the case for primary monosomes, the cytologically unbalanced gametes from the chain are not transmitted by the male gametes.

In determining linkage of \( R, Lc, N \) with chromosome 3, the critical ratios would occur with either \( R \) or \( N \), depending on which were in haplo-arm 4. If one were in haplo-arm 4, then the data of the backcross of the \( F_1 \) chain of three as female to DPL-14 would show only diplo-dominant (26II) and haplo-recessive (24II + III) for that particular locus. In the reciprocal backcross only dominant phenotypes would be recovered. However, the data in Table 2 show that the expected results were not obtained for either \( R \) or \( N \). Both dominants and recessives for the two loci were found in the two cytological classes from the backcross of the \( F_1 \) chain of three \( \times \) DPL-14. If linkage were involved only diplo-dominants and haplo-recessives would have been recovered for one of the two loci. In the reciprocal backcross, again, both dominants and recessives were recovered instead of only dominants, as expected in case of linkage. Therefore, we must conclude that linkage group I is not in chromosome 3.

Flower petals of DPL-14 are cream in color. The petals of Texas 586 are full yellow (\( Y_1 \)), whereas the color of petals of Z2229, which carried the chain of
three, is pale yellow with intensification of yellow around the "throat" of the petals. Z2229 originated from selfed seed of the second backcross of the three-species hybrid 2 (G. arboreum yellow × G. thurberi cream) × Upland cream; therefore, it can be assumed that the pale yellow petal color in Z2229 came from G. arboreum.

The data given in Table 2 show that in both backcrosses and the control, only full yellow and pale yellow flower types were recovered. In case of the chain of three chromosomes, had the pale yellow locus been located in either haplo-arm 3 or haplo-arm 4, that arm would have been replaced in the F₁ by its homologous segment carrying the Y₁ locus of Texas 586. The F₁ chain of three would be hemizygous for Y₁. Consequently, on backcrossing the F₁ chain of three as female to DPL cream, all diplo-plants should have had yellow petals and all haplo-plants should have had cream petals. In the reciprocal backcross only yellow petals should have been recovered. It is obvious from the data given in Table 2 that this was not the case. In the backcross in which the F₁ chain of three was the female, both yellow and pale yellow types were recovered in the two cytological classes. In the reciprocal backcross, yellow and pale yellow types occurred. Therefore, Y₁ is not in haplo-arm 3 or haplo-arm 4. When it became apparent that Y₁ was not associated with the haplo-segments, no attempt was made to separate accurately the yellow flowered types from the pale yellows, since the distinction of the two was not always as clear as that commonly observed between yellow and cream; this accounts for the low numbers in the pale yellow class in Table 2.

In the case of pollen color, Table 2 shows that yellow (Pa) and cream pollen (pa) plants were recovered in both cytological classes in the F₁ × DPL backcross. Also, both phenotypes were recovered in the reciprocal backcross. If the Pa locus were in one of the haplo-segments, then, in the backcross of the F₁ × DPL, all 26 II plants should have had yellow pollen and all 24 II + III plants should have had cream pollen. In the reciprocal backcross, only plants with yellow pollen should have been recovered. Obviously the Pa locus is not in haplo-arm 3 or haplo-arm 4. Monosomic analysis revealed that Pa was not in chromosome 2 which includes arm 3 (Table 1).

**SUMMARY**

Ten dominant mutant genes were tested for association with six primary monosomes (4 of A subgenome and 2 of D subgenome), and five dominant mutant
genes were tested for association with one tertiary monosome (A subgenome) in *Gossypium hirsutum*. The results are as follows: (1) linkage group IV, *H*, *Lc*, of the A subgenome is in chromosome 6 of the A subgenome; (2) linkage group I, *R*, *Yg*, *cl*, *lc*, *N*, of the A subgenome is not on chromosomes 1, 2, 3, 4 nor 6 of the A subgenome; (3) *Pa*, which is in the A subgenome, is not on chromosomes 1, 2, 4, 6, nor arm 4 of chromosome 3; (4) *Yl*, which is in the A subgenome, is not on chromosomes 4, 6, arm 4 of chromosome 3, nor arm 3 of chromosome 2; (5) the D subgenome linkage groups II, *Lo*, *Lg*, and III, *cl*, *R*, *Yg*, *Dw*, are on neither chromosome 17 nor 18 of the D subgenome.

**LITERATURE CITED**


