

# Twin Spots on Leaves of Homozygous Cotton Plants

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**M**OSAIC patterns caused by various types of genetic changes in somatic tissues have been observed in the form of single and twin spots in a number of organisms. Stern<sup>16</sup> observed single yellow (*y*) spots and singed (*sn*) spots and twin yellow-singed spots on the wild-type body of a heterozygous female ( $\frac{+ sn}{y +}$ ) in *Drosophila melanogaster*. He also observed three types of single spots on the bodies of  $\frac{y sn}{++}$  females. The expected yellow and singed spots, and the yellow and the singed single spots occurred from the separation of the linked *y* and *sn* genes by somatic crossing over in the four-strand stage.

Jones<sup>15</sup> reported paired and unpaired spots involving several genes determining color and texture differences in the triploid aleurone layer of the maize endosperm. The aleurone tissue was heterozygous *Aaa* or *AAa*, depending on the genotype of the pollen and seed parents for each gene considered. Nondisjunction, deletion, incomplete fertilization, and mutations were given as possible explanations for the occurrence of single spots. A hypothesis of somatic crossing over appeared to fit many of the observations on twin spots, but Jones held some reservations on the acceptance of that explanation.

Vig and Paddock<sup>22</sup> observed apparent somatic recombination at the *Y<sub>11</sub>* locus in soybeans. Incomplete dominance was expressed by *Y<sub>11</sub>*, in that *Y<sub>11</sub>Y<sub>11</sub>* was normal green, *Y<sub>11</sub>y<sub>11</sub>* was light green and *y<sub>11</sub>y<sub>11</sub>* was greenish yellow. Unpaired and paired spots of normal green (*Y<sub>11</sub>Y<sub>11</sub>*) and greenish yellow (*y<sub>11</sub>y<sub>11</sub>*) were observed on the two simple leaves and the first compound leaf of a heterozygous *Y<sub>11</sub>y<sub>11</sub>* light-green plant. Treatment with mitomycin C, a chemical known to increase somatic crossing over in a number of organisms, increased both single and twin spots but dispropor-

tionately increased the twin spots. They suggested that twin spots arose from somatic crossing over, and in the absence of mitomycin-C single spots may arise from other chromosome aberrations. Vig<sup>21</sup> correlated mitotic irregularities with twin spot occurrences, and related them to possible somatic crossing over.

Ross and Holm<sup>17</sup> noted a similar response in heterozygous *aurea* (*A+*) mutant tomato plants. Single and twin spots with the apparent genotypes *++* green and *AA* yellow were observed on one or more leaves per plant. They indicated a similar mechanism of somatic crossing over as described by Stern<sup>16</sup> may have been responsible, but they were doubtful of this explanation because of the lack of observed somatic pairing in plants.

Twin spots or sectors were observed in apples and illustrated by Dahlgreen<sup>7</sup>. Because of the known heterozygosity in apples, somatic segregation seems to explain the phenomenon.

In maize, a medium variegated allele *P<sup>rr</sup>* determines irregular red striping in the pericarp and cob. This allele mutates frequently to the stable red (*P<sup>rrr</sup>*) allele. A hypothesis by Brink and Nilan<sup>4</sup> and Brink<sup>3</sup> explained the variegated allele as a complex locus (*P<sup>rr</sup>*). When *Mp* was transposed to another site, the *P<sup>rr</sup>* locus expressed full red color. The *P<sup>rr</sup>-Mp* locus with another dose of *tr-Mp* on the same chromosome produced the light variegated kernels.

Greenblatt and Brink<sup>12</sup> studied the medium variegated pericarp allele in stocks heterozygous for stable, colorless, pericarp alleles *P<sup>rrr</sup>* or *P<sup>rr</sup>* (white cob and red cob, respectively). In these stocks the medium variegated pericarp was dominant and twin spots of red and light variegated pericarp areas occurred frequently. Linkage data were presented to support a hypothesis of twin mutations occurring simultaneously to produce the twin spots. It was postulated that in the chromosome carrying the *P<sup>rr</sup>-Mp* locus, giving *P<sup>rrr</sup>-Mp/P<sup>rr</sup>-Mp*, the *Mp* portion on one chromatid would be transposed to another site on an unreplicated portion of the same chromosome. The new *tr-Mp* (*t* = transposed) locus would replicate giving *P<sup>rr</sup>* and *tr-Mp* at different loci on one chromatid, and expressing the red phenotype in one daughter cell. The other

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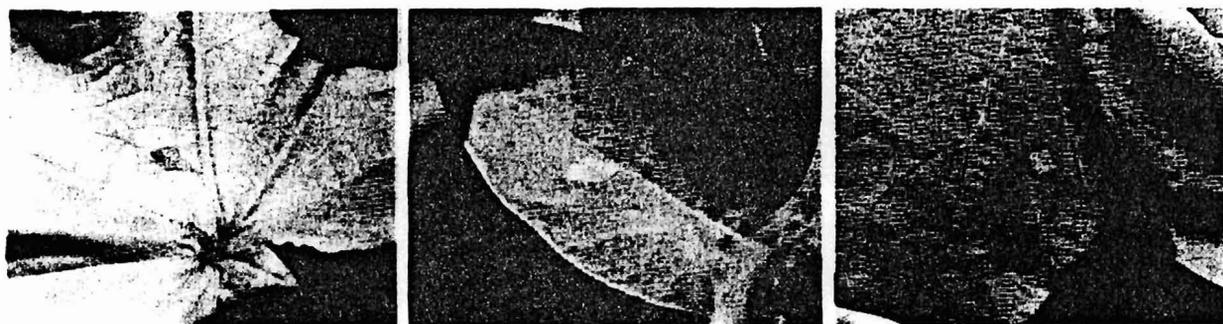


FIGURE 1—Spots on virescent  $r:r$  leaf tissue. Left—green spot darker than virescent; note the yellowing

effect of sunlight. Center—yellow spot lighter than virescent. Right—yellow-green twin spot.

sister chromatid with  $P^{rr}-Mp$  and  $tr-Mp$  at the same loci on the sister chromatid after segregation, would give the light variegated phenotype in the other daughter cell. Further division of the two daughter cells would give rise to tissues that were either red or light variegated.

Imai and Kanna<sup>14</sup> found that three phenotypes of *Portulaca grandiflora* L., namely, orange, striped yellow, and striped cream were homozygous dominant ( $CC$ ), heterozygous ( $Cc$ ) and homozygous recessive ( $cc$ ), respectively. Within the striped yellow ( $Cc$ ) plants, orange ( $CC$ ) sectors frequently occurred. In selfed homozygous striped cream ( $cc$ ) plants a low percentage of orange ( $CC$ ) plants (1.5 percent) and 13.5 percent striped yellow ( $Cc$ ) plants were observed in the progenies. Sectors of striped yellow and orange often were observed in striped cream ( $cc$ ) plants. The orange plants did not revert to the striped yellow or striped cream phenotypes. These observations were explained by a high mutation frequency of  $c$  to  $C$ .

Brown<sup>6</sup>, in a review of the topic, states that somatic pairing of homologous chromosomes is apparently a more common phenomenon than was formerly thought. It has been observed in insects, fungi, and higher plants. In many cases genetic recombination of marker genes has been detected, as stated above. It has also been determined that in some plants of allopolyploid origin, homoeologous chromosomes may pair meiotically in the absence or mutation of specific genetic control of pairing<sup>10</sup>. Somatic association of chromosomes in mitotic cells has also been shown in *Triticum aestivum*<sup>11</sup> and in *Avena*<sup>18</sup>. Endrizzi<sup>9</sup> and Barrow<sup>1</sup> detected association of chromosomes of the A and D genomes in haploids of cotton. Brown<sup>7</sup> observed an average of seven to nine paired chromosomes per cell during pachytene in a haploid of *Gossypium hirsutum*. However, as meiosis progressed, the associated chromosomes separated without chiasmata. It was

assumed that the associated chromosomes were homoeologues from the A and D genomes.

#### Materials and Observations

The expression of virescent leaf color in cultivated cotton *Gossypium* sp. is inherited as a recessive characteristic. The normal leaf color is determined by dominant genes. A yellow-green virescent ( $v_7:v_7$ ) strain of *G. barbadense* L. that produces a high frequency (about 50 percent) of haploid ( $n=2x=26$ ) plants was obtained from E. L. Turcotte of Phoenix, Arizona, as an  $F_3$  plant selection. The  $F_1$  was grown in the greenhouse and selfed and plants of the  $F_2$  were grown in the field and greenhouse during 1971. Of the surviving plants in the field, 261 were diploid and 120 were haploid. Only diploid plants were selected for greenhouse culture. As expected, all diploid plants were virescent, indicating complete homozygosity ( $v_7:v_7$ ), and all haploid plants were virescent ( $v_7$ ).

During the growing season, three types of leaf spots were observed. Green spots darker than the virescent tissue, yellow spots lighter than the virescent, and green and yellow twin spots or adjacent areas of the green and yellow colors (see Figure 1). The diploid  $F_2$  plants in the greenhouse were also examined and found to have spots.

Table I gives the number of plants and spots observed in the field and greenhouse. Table II gives the frequency of spots per leaf based only on leaves with spots. There were approximately five times as many spots per leaf in the greenhouse as were observed in the field. A possible cause of more spots in the greenhouse was that greenhouse summer temperatures averaged about 10°C warmer than the field. Also the more intense sunlight in the field may have washed out some spots, particularly yellows, before they were recorded. The increase of each type of spot in the greenhouse appeared to be approximately equal in magnitude.

A close observation of haploid plants revealed all three types of spots present, with a lower proportion of twin spots and a higher proportion of single yellow spots (Table III). There was a lower percentage of haploid plants showing spots than diploid plants.

Cytological examination of PMC's of nine diploid plants with spots revealed no irregularities in meiosis. Examination of PMC's in meta-anaphase<sup>1</sup> of two haploid plants with twin spots showed 26 univalents lacking any apparent bivalent formation.

### Discussion

The reports of spotting and variegation in *Drosophila*<sup>10</sup>, maize<sup>15</sup>, tomato<sup>17</sup>, soybeans<sup>22</sup>, and apples<sup>7</sup>, involved heterozygous material and the twin spots were thought to be the result of somatic crossing over and/or segregation of homologous chromosomes. That is, the twin spots were interpreted as homozygous sectors derived from heterozygous cells. In the above cases, except for *Drosophila*<sup>10</sup>, incomplete dominance was noted in each instance and it was necessary to distinguish between the three genotypes *AA*, *Aa*, and *aa* in the sectors.

The spotting described in this report was in a strain of cotton that responded as if it were homozygous. Apparently heterozygous sectors were derived from homozygous cells. If one considers somatic crossing over in the  $v_1v_1$ ; cotton plants as the cause of spots, then some unknown type of heterozygosity that would have no apparent phenotypic effect must exist in the  $v_1v_1$ ; plants. One possibility would be a pseudo-allelic condition where somatic crossing over within the allele between two minute mutations could give the segregation observed. Another possibility would be a pericentric inversion with the centromere lying between two hypothetical color-influencing genes located on each arm of the chromosome beyond the inversion. A crossover in the inverted region would give duplications and deficiencies of the two color genes that may be expressed as twin spots. In the above cases, the heterozygous tissue would be phenotypically indistinguishable from the homozygous tissue and any phenotypic change would be the result of somatic crossing over. Inversion and somatic crossing over between homologues are unlikely, however, for two reasons: 1) The expected frequency of heterozygotes would be reduced by self-fertilization from 50 percent in the  $F_2$  to 6

percent in the  $F_3$ . It was observed that among 261 diploid plants, 236 (90 percent) expressed spots in the  $F_3$  generation of selfing. 2) All three types of spots were found in haploid plants, which rules out crossing over or unequal crossing over between homologous chromosomes.

The high frequency of spotting and the general low frequency of mutation makes an unlikely hypothesis that two different mutations (to green and yellow) occurred simultaneously in adjacent cells. However, an unstable  $v_1$  locus functioning similarly to the  $p''$  locus in maize with a transposable modulator as described by Greenblatt and Brink<sup>12</sup> may provide a workable model. We also observed twin spots in a virescent  $v_1v_1$  strain of *G. hirsutum* L. indicating the general instability of the virescent loci.

The best explanation for the spotting is based on information obtained by Turcotte and Feaster<sup>20</sup>, combined with the results of somatic crossing over of homoeologous chromosomes. They found that in two different cotton strains the genes for virescent behaved as simple recessives. In a test for allelism they noted that the  $F_1$  of the two strains was virescent and the  $F_2$  segregated 5 green : 6 virescent : 5 yellow, a modified dihybrid ratio. Ap-

Table II. The frequency of spots per leaf (counting only leaves with spots) in diploid plants in the field and greenhouse

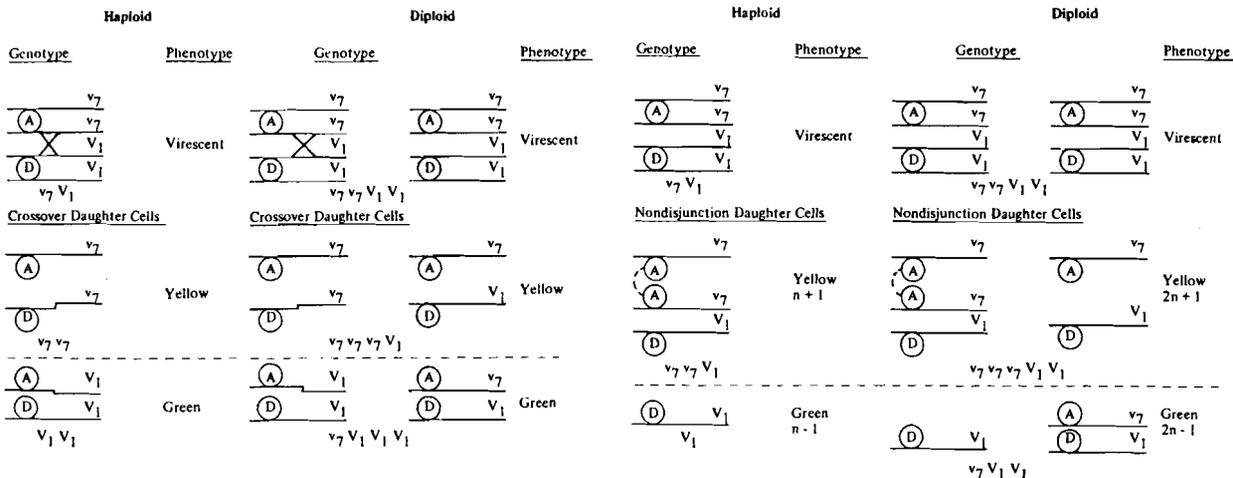
	Green spots	Yellow spots	Twin spots	Total
Field	0.51	0.30	0.33	1.14
Greenhouse	2.85	1.12	2.08	6.05
Ratio: Spot frequency in field to greenhouse	1:5.58	1:3.73	1:6.30	1:5.30

Table III. Haploid  $v_1$  plants with single green, single yellow, and twin spots observed in the field

Total plants	No. plants with spots	No. leaves with spots	Green spots	Yellow spots	Twin spots	Total spots
120	38	47	20	30	2	52

Table I. Diploid  $v_1v_1$  plants with single green, single yellow, and twin spots observed in the field and greenhouse

	Total plants	No. plants with spots	No. leaves with spots	Green spots	Yellow spots	Twin spots	Total spots
Field	261	236	824	421	252	270	943
Greenhouse	75	59	213	607	239	443	1289



**FIGURE 2**—A model of somatic pairing and crossing over between homoeologous chromosomes of the A and D genomes of haploid and diploid cotton plants. It is assumed that the  $V_1$  and  $v_7$  loci are on homoeologous chromosomes; however, it is not known in which genome the recessive gene resides. The segregation of the genes  $v_7$  and  $V_1$  leading to the formation of yellow and green chimeral spots derived from virescent tissue is shown. The chromosomes of the respective genomes are indicated by the letters within the circles representing the centromeres. The "X" represents an exchange between the chromatids of the homoeologous chromosomes.

parently a dosage effect was responsible for the unusual dihybrid segregation. The two virescent parental strains were suspected to be genotypically  $v_1 v_1 V_7 V_7$  and  $V_1 V_1 v_7 v_7$ . In the  $F_2$  three to four dominant genes (0–1 recessives) would give green color; two dominant genes (2 recessives) would give the virescent color; and one or no dominant genes (3–4 recessives) would give a yellow color.

Cultivated cotton, an allotetraploid, is composed of two subgenomes A and D<sup>2</sup>. Holder<sup>13</sup> established linkage values for  $gl_2-ne_1$  in the A genome and for the duplicate linkage of  $gl_3-ne_2$  in the D genome. Rhyne<sup>16</sup> similarly established the duplicate linkage of  $R_2-Yg_2$  in the A genome and  $R_1-Yg_1$  of the D genome. This indicates that duplicate homoeologous linkage groups are found in the two genomes.

As stated above, Brown<sup>5</sup> reported an average of 7–9 paired chromosomes of equal length and differing in heterochromatin at pachytene in *G. hirsutum* haploids. These bivalents were apparently A–D homoeologous chromosomes. Endrizzi<sup>18</sup> and Barrow<sup>1</sup> also observed A and D associations at pachytene in haploid cotton plants.

Endrizzi<sup>19</sup> states that chromosomes of the different genomes of *Gossypium* are the same length in the dispersed interphase state, but due to differential coiling, the characteristic large A genome and small D genome chromosomes are observed during cell division. He suggests this differential rate of

**FIGURE 3**—Genotypic and phenotypic effects of mitotic nondisjunction resulting in yellow and green tissue. It is assumed in the haploid and diploid plants that the chromatids of the chromosome bearing the  $v_7$  locus are not disjoined, producing aneuploid daughter cells and altered genotypes and phenotypes.

coiling is the reason for the diploid-like behavior of allotetraploid cottons.

The above evidence of homology between genomes and the reported duplicate linkage groups supports the hypothesis that the  $V_1$  and  $V_7$  loci are on homoeologous chromosomes. Somatic pairing of the homoeologues, with crossing over between the centromere and the  $V$  locus would explain the single and twin spots observed in both the haploid and diploid plants.

Figure 2 illustrates a model of crossing over between homoeologous A and D chromosomes in haploid and diploid plants assuming virescent genotypes are  $V_1 v_7 (n)$  and  $V_1 V_1 v_7 v_7 (2n)$ , and that the loci are on homoeologous A and D genome chromosomes. Which gene is in which genome is unknown, although we have, for purposes of illustration, arbitrarily placed the  $v_7$  gene in the A genome. In the haploid and diploid plants an A–D pair crosses over and in the diploid plants the other A–D pair undergoes normal mitotic division. The daughter cells after somatic crossing over and mitotic segregation in the haploid plants would be  $v_7 v_7$  yellow and  $V_1 V_1$  green. In the diploid plants daughter cells would be  $v_7 v_7 v_7 V_1$  yellow and  $v_7 V_1 V_1 V_1$  green as a result of A–D crossing over. Single yellow or green spots would result from the same mechanism as twin spots by failure of one or the other of the daughter cells to divide, after somatic crossing over occurs.

Cytogenetically, somatic crossing over between homoeologous chromosomes will produce a structural change identical to a reciprocal translocation. If chromosome pairing and crossing over of this type occurred in floral meristems, some sterility

may be explained. It would also be expected that multivalents might be observed at meiosis.

Another explanation for the occurrence of twin spots and single spots may be mitotic nondisjunction in either of the homoeologous chromosomes. A color change in mitotic daughter cells would be expected due to changes in the genotype resulting from nondisjunction of chromatids of either an A genome chromosome or a D genome chromosome. For example, Figure 3 illustrates nondisjunction in an A genome chromosome. In haploids nondisjunction would produce  $n + 1$  and  $n - 1$  cells, with the possibility of lower viability for the latter. Figure 3 is drawn to show the  $n + 1$  cell as yellow and the  $n - 1$  cell as green. Lower viability of the  $n - 1$  cells may account for the observed higher frequency of yellow spots in haploids (Table III). Nondisjunction in diploid cells would result in  $2n + 1$  and  $2n - 1$  daughter cells, and no decrease in viability would be expected. The reason for the higher frequency of green spots in the diploids is unknown (Table I).

The authors, on the basis of more recent information, favor the somatic crossing over hypothesis for the cause of twin spots in cotton.

### Summary

Single yellow, single green, and twin yellow-green spots were observed in apparently homozygous virescent cotton plants. The spots observed in both haploid and diploid plants were best explained by somatic crossing over between homoeologous A and D genome chromosomes, or mitotic nondisjunction of either an A or D genome chromosome, plus a dosage effect of independently segregating duplicate genes in the two genomes.

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