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Inheritance of the H_1, H_2, and Sm_2 Genes in Cotton[1]

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ABSTRACT

Based on early genetic studies of inter- and intra-specific crosses in cotton, the genes for increased plant pubescence, H_1 and H_2 are independently inherited. More recent studies with aneuploids of *Gossypium hirsutum* L. showed that the H_1 and H_2 genes and the Sm_2 gene (that removes trichomes from the plant) all are located on chromosome 6, and that the H_2 and Sm_2 are located in the long arm of the chromosome. The H_2 gene was mapped 4 units from the centromere. Based on the latter data, the three genes may either be closely linked or alleles. In this study, we used aneuploids to show that the H_1 and Sm_2 genes also are located 4 map units from the centromere in the long arm of chromosome 6. In addition to the cytogenetic approach, we also studied the F_2 populations of the three crosses of $H_1 \times H_2$, $H_1 \times Sm_2$, and $Sm_2 \times H_2$ and found that the three genes segregated as alleles. In view of these results, a revised nomenclature for the smooth-hairiness genetical system in cotton is needed.

Additional index words: ***Gossypium hirsutum*** **L.,** ***Gossypium barbadense*** **L.,** ***Gossypium tomentosum*** **Nutt. ex Seem., Genetics, Cytogenetics, Aneuploids.**

THE presence of pubescence on plants of the cultivated species of *Gossypium* is important in relation to insect control and cotton dust in textile mills. In view of this, the relationships of genes determining pubescence development is important to breeders of cotton cultivars.

In Upland cotton, *Gossypium hirsutum* L., six dominant genes have been reported to increase the amount or density of pubescence or plant hairs on the vegetative parts. Two of these were designated as H_1 and H_2 by Knight (1952) who reported that they are independently transmitted. Independence in inheritance of the two factors was also reported by Knight and Sadd (1953) and Ramey (1962).

Saunders (1961) reviewed the early genetic studies involving the H_1 and H_2 genes and noted that the results gave conflicting conclusions. In his genetic studies involving crosses of the two hairy genes with glabrous forms, he reported that, except for one of the lines (T611) with the H_2 gene, the F_2 families gave a continuous type of distribution.

We reviewed Knight's (1952) data on the segregation of H_1 and H_2 genes, which initially were derived from interspecific hybridization of the wild allotetraploid *G. tomentosum* Nutt. ex. Seem. and *G. barbadense* L. We noted that much of his data could be interpreted as the result of segregation of alleles at a single locus. In fact, the F_2 of the third backcross contained only hairy types and in a ratio of 1:2:1 for different levels of hairiness. This would be expected if H_1 and H_2 are alleles which condition different levels of hairiness.

Endrizzi and Kohel (1966) reported that the H_2 gene is located four map units from the centromere in the long arm of chromosome 6 of *G. hirsutum* L. In tests with monosomic lines of *G. hirsutum*, Endrizzi (1975) reported that the H_1 gene and a gene for smooth plant, Sm_2 (Lee, 1968), also reside in chromosome 6, and that the Sm_2 gene is located in the long arm of the chromosome. Thus, the H_1, H_2 and Sm_2 could be alleles or linked genes in chromosome 6.

A study was conducted to determine the genetic relationship of the above three genes. Reported herein are cytogenetic studies with monotelodisomes of chromosome 6 and a genetic analysis of F_2 populations which show that the H_1, H_2 and Sm_2 genes are allelomorphs.

MATERIALS AND METHODS

Cultivars of *G. hirsutum* normally have a moderate quantity of pubescence on plant parts, typical of that in the standard line Texas marker-1 (TM-1) (Kohel et al., 1970), and are classified as normal hairy. We used TM-1 as our standard for normal hairy phenotype and as the normal hairy line in backcrosses with F_1 Sm_2 sm_2.

Plants with either the H_1 or H_2 genes have more or longer hairs or both and are referred to as hairy. Plants having the Sm_2 gene are essentially devoid of pubescence and are classified as smooth. The three lines carrying these genes that were used in this study are described in the following.:

H_1H_1, *Hirsute MU8b.* An American Upland variety homozygous for H_1H_1 that was originally selected by J. B. Hutchinson in India. The line also carries genes that intensify the effect of H_1 (Knight, 1952). MU8b, designated as AG113 in the Tucson cotton collection, was provided by Dr. R. J. Kohel of Texas A&M University after receiving it from Dr. J. H. Saunders. H_1 produces dense pubescence on leaves, bracts and stems but not on bolls.

H_2H_2, *Pilose, Texas 586.* The mutant was originally isolated from an Upland cross and described and designated as T611 by Simpson (1947). T586 is a multiple dominant stock developed at the Texas Agricultural Experiment Station. H_2 produces pubescence in leaves, bracts, stems, and bolls.

Sm_2Sm_2, *Smooth.* A line homozygous for Sm_2 was isolated by Lee (1968) from WH-219, an ultrasmooth house-yard accession of *G. hirsutum* from Nicaragua. The stock was received from Dr. J. Lee of North Carolina State University and designated as AG108 in the Tucson cotton collection. Sm_2 removes the pubescence from all plant parts except for a fringe of hairs along the margins of the leaves.

For our cytological studies, we used two lines with telocentric chromosomes for the long and short arms of chromosome 6, des-

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ignated, respectively, as monotelodesomic-6La and -6Sa. The genotypes of the two lines are described in the following:

Monotelodisomic-6La. Monotelodisomic-6L plants have a standard chromosome and a telocentric chromosome for the long arm of chromosome 6. The line is normal or recessive for pubescence and hemizygous for the Brymer Brown lint color, Lc_2. From self-pollination of the monotelodisomic, the disomic plants will be Lc_2Lc_2 whereas the monotelodisomic plants will be Lc_2/-(hemizygous). The stock originated from a single plant selection out of the cytogenetic study involving H_2Lc_2 linkage reported by Endrizzi and Kohel (1966). Monotelodisomic-6L plants are recognized from the disomes by their short-fruiting branches (Endrizzi and Ramsay, 1979).

The gene for Brymer Brown lint color was shown in monosomic tests to be located on chromosome 6 (Endrizzi, 1963). Later, Endrizzi and Kohel (1966) reported in linkage analysis with monotelodisomic lines that this brown lint gene is located in the short arm of chromosome 6 and is 22 map units from the H_2 gene.

In a study of several lines with brown lint, Stephens (1955) assigned the gene symbol, Lc_2, to the Brymer Brown lint color. Endrizzi and Kohel (1966) had concluded from their study that Brymer Brown was not an allele at the Lc_2 locus; however, a re-evaluation of the current data indicate that Brymer Brown is an allele at the Lc_2 locus.

Because the monotelodisomic-6L line had the Lc_2 gene, which is hemizygous, this enabled us to determine the genetic linkage of Lc_2 and Sm_2 as well as linkage of Sm_2 and the centromere in the cross with Sm_2 Sm_2. Two types of F_1 progeny are produced by crossing monotelodesomic -6L as female parent to AG108 Sm_2 Sm_2. One will be disomic with the genotype of sm_2 Lc_2/Sm_2lc_2 and the other will be monotelodisomic-6L with the genotype of sm_2-/Sm_2 lc_2 (the dash represents the hemizygous condition of the lc_2 locus: see Table 2). Endrizzi (1975) had reported, without presenting data, that the Sm_2 was located in the long arm of chromosome 6.

Monotelodisomic-6Sa. Monotelodisomic-6S plants have a standard chromosome and a telocentric chromosome for the short arm of chromosome 6. The stock is recessive for alleles determining pubescence and lint color. Monotelodisomic-6S plants can be recognized by the expression of the hemizygous genotype of the genes affecting pubescence.

As was pointed out above, outcrossing the monotelodisomics as females with normal lines will produce progeny consisting of two cytotypes: monotelodisomics and disomics. Although the monotelodisomics can be identified phenotypically by their associated plant characters, cytological analysis was used to verify their identity in the present study. The procedure was done by routine fixation of young flower buds in 7 alcohol: 3 acetic acid solution and subsequent analysis of the meiotic Metaphase I stage by the iron-propionic acid squash procedure.

The two monotelodisomic lines were crossed to AG108 Sm_2Sm_2 to verify the arm location of the Sm_2 gene and to obtain the genetic distance of Sm_2 from the centromere and the Lc gene. Monotelodisomic-6L was crossed as female to MU8b H_1H_1 to determine the amount of recombination between H_1 and the centromere. The F_1's were used to obtain testcross data for analysis for the association of characters.

RESULTS AND DISCUSSION

Genetics of Pubescent (Hirsute), H_1, Gene

In monosomic tests, H_1 of MU8b was found to reside on chromosome 6 (Endrizzi, 1975) in the analysis of F_2 and testcross progeny. In that report, the 2n-1 mono-6 pubescent F_1 was crossed reciprocally to the standard tester TM-1 h_1h_1. Thirty-three plants were grown and scored in the testcross of TM-1 × F_1 mono-6 H_1 as male, and all were pubescent and disomic, establishing that H_1 is on chromosome 6. Not reported by Endrizzi (1975) were the results of the reciprocal testcross, which are presented here. The reciprocal testcross, in which both disomic and monosomic progeny are normally recovered, consisted of 25 disomes and four monotelodisomics. The 25 disomes were pubescent and the four monosomes were nonpubescent or normal hairy. The results observed in the reciprocal testcrosses were exactly as expected for association of a marker and a monosomic chromosome. In addition to the above plants, there was one nonpubescent plant that apparently was monotelodisomic for the short arm of chromosome 6 because the plant had short sympodia and an overall plant habit that was typical of monotelodisomic-6L plants. The telocentric chromosome apparently arose from meiotic misdivision of the mono-6 chromosome. The recovery of a plant that was deficient for the long arm of chromosome 6 and lacking the H_1 allele proves that the H_1 locus is located in the long arm of that chromosome. This fact is supported by data given in Table 1.

Table 1. Segregation of H_1h_1 in testcrosses of F_1 monotelodisomic 6-L and F_1 disomics in cotton.

Genotypes of testcrosses	Number of progeny in genotype classes: 2n H_1	2n h_1	25″ + tl″ H_1	25″ + tl″ h_1	Total
$Sm_2Sm_2 \times \frac{H_1}{h_1}$ †	216	3	0	31	250‡
$Sm_2Sm_2 \times \frac{H_1}{h_1}$	12	13			25

† The lines illustrate a normal (upper) and a telocentric (lower) chromosome. The dots represent the approximate position of the centromere.
‡ Percent recombination H_1-centrometer = 1.2 ± 0.69; percent male transmission of telocentric = 5.2.

Because the above data indicated that the h_1 locus is located in the long arm of chromosome 6, a monotelodisomic-6L, h_1h_1 plant was crossed with MU8b, H_1H_1. In this cross the normal chromosome of the monotelodisomic bivalent will carry the H_1 allele and the telocentric homolog will carry the h_1 allele.

The F_1 monotelodisomic-6L plants were crossed as males to Sm_2Sm_2 to determine if H_1, like H_2, is located in the long arm and tightly linked with the centromere. When a monotelodisomic plant is used as the male parent, a majority of the recovered gametes will have the normal haploid set of chromosomes and a minority of the gametes will have the telocentric chromosome. Whether the normal or the telocentric chromosomes carries either the H_1 or the h_1 allele is a function of the distance of the locus from the centromere.

Table 1 shows that 216 of the 219 disomic plants were pubescent (H_1h_1) and that the 31 monotelodisomic plants were nonpubescent (h_1h_1), both of which are parental chromosomal types. This means that the h_1 locus is tightly linked with the centromere of chromosome 6. The three disomic plants that are nonpubescent are recombinants, giving a recombination value of 1.2 ± 0.69% between the h_1 locus and the centromere.

A disomic F_1 also was crossed to Sm_2Sm_2 to check for normal segregation of H_1 and h_1 alleles. Segregation was approximately 1:1 as shown in Table 1.

In a similar study involving the Pilose gene, H_2, Endrizzi and Kohel (1966) reported that the H_2 locus is located in

Table 2. Segregations of Sm_2sm_2 in testcrosses of F_1 monotelodisomic-6L and segregation of sm_2Lc_2/Sm_2lc_2 in testcross of F_1 disomics in cotton.

Cross no.	Genotypes of testcrosses†	Number of plants in genotype classes: Normal sympodia (disomes)		Short sympodia (monotelodisomes)		Total
		Smsm	*smsm*	*Smsm*	*smsm*	
1	$sm_2sm_2 \times \frac{Sm_2lc_2}{sm_2}$	197	8	0	35	240‡
		Percent recomb. Sm_2-centromere = 3.33 ± 0.30§				
		Percent male transmission of telocentric = 7.0				
		SmSm	*Smsm*	*SmSm*	*Smsm*	
2	$Sm_2Sm_2 \times \frac{Sm_2lc_2}{sm_2}$	129	4	1	9	143‡
		Percent recomb. Sm_2-centromere = 3.50 ± 0.24				
		Percent male transmission of telocentric = 14.6				
		Smsm Lclc	*Smsm lclc*	*smsm Lclc*	*smsm lclc*	
3	$sm_2Lc_2/Sm_2lc_2 \times sm_2lc_2/sm_2lc_2$	48	239	217	58	562‡
		Percent recomb. Sm_2Lc_2 = 18.86 ± 1.65				

† The lines of the monotelodisomic F_1's represent normal (upper) and a telocentric (lower) chromosome. The dots represent the approximate position of the centromere.
‡ Chi-square values were significantly different from random segregation.
§ Pooled percent recomb. Sm_2-centromere (cross 1 + 2) = 3.40 ± 0.24.

the long arm of chromosome 6 and 0.99 ± 0.49 map units from the centromere. The two recombination values observed with H_1 and H_2 are not separable at the 68% confidence level.

Because both H_1 and H_2 are located in the long arm of chromosome 6 and map approximately 1cM from the centromere (uncorrected for reduction in recombination in the proximal region, Endrizzi and Kohel, 1966), we conclude that H_1 and H_2 are alleles at a common locus rather than genes at independently segregating loci as previously concluded by Knight (1952), Knight and Sadd (1953), and Ramey (1962). In the tests for allelism, reported below, the two factors behaved as alleles.

Cytogenetic Studies with Sm_2

Monosomic and telosomic analysis revealed that the Sm_2 gene also is located in chromosome 6 (Endrizzi, 1975). In that report, the results of the test cross which established that Sm_2 is located in the long arm were not reported. These results are reported below.

In the cross of Sm_2Sm_2 as male to monotelodisomic-6L, both the disomic and monotelodisomic F_1 plants were intermediate-hairy indicating that both were heterozygous (Sm_2sm_2). In the cross of Sm_2Sm_2 as male to monotelodisomic-6S, however, the disomic F_1 plants were intermediate hairy and the monotelodisomic-6S F_1 plants were almost as smooth as the Sm_2Sm_2 parental line. The difference in plant hair expression in the two cytotypes in this second cross can be attributed to differences in their genotypes. The disomic F_1's were assumed to have the heterozygous genotype of Sm_2sm_2, whereas, the monotelodisomic F_1's were assumed to have the hemizygous genotype of Sm_2/-. This means that Sm_2 is located in the long arm. Because plants of the sm_2sm_2 genotype are normal pubescent, the absence of the sm_2 allele in the monotelodisomic-6S F_1's would account for the near-smooth phenotype.

Since Sm_2 apparently is in the long arm of chromosome 6, the monotelodisomics-6L F_1's were crossed as males to TM-1 sm_2sm_2 and to AG108 Sm_2Sm_2 so as to map the sm_2 locus in relation to the centromere. The monotelodisomic-6L parent was hemizygous for the Lc_2 allele. Therefore, in crossing with Sm_2Sm_2, the F_1 monotelodisomic-6L plants were heterozygous Sm_1sm_2 and hemizygous lc_2/-, whereas the F_1 disomic sibs were heterozygous sm_2Lc_2/Sm_2lc_2 (Table 2). The disomic F_1 sibs were crossed to TM-1 to determine the map distance bewteen Sm_2 and Lc_2 loci. The testcross segregation data for both the F_1 monotelodisomic-6L and F_1 disomics are given in Table 2.

The data in Table 2 show that in the two populations obtained from testcrossing the F_1 monotelodisomic-6L, Sm_2sm_2 as female to sm_2sm_2 and Sm_2Sm_2, a majority of the progeny inherited the Sm_2 allele from the F_1 parent. The Sm_2 allele in both F_1's was transmitted almost to the exclusion of the sm_2 allele. This was expected since the Sm_2 allele is carried on the long arm of the standard chromosome and closely linked with the centromere. The mode of segregation in these two tests was very similar to that in the testcross of $Sm_2Sm_2 \times F_1$ monotelodisomic, H_1h_1. (Table 1).

In testcross 1, the only progeny carrying recombinant chromosomes are the eight disomics with the sm_2sm_2 genotype, which are the result of recombination between the locus and the centromere. Thus, the percent recombination between the Sm_2 locus and the centromere is 3.30 ± 0.30.

In testcross 2, the testcross progeny having recombinant chromosomes are the 12 Sm_2sm_2 disomes and the one Sm_2Sm_2 monotelodisome, giving a recombination value of 3.50 ± 0.24% between Sm_2 and the centromere.

Pooling the data of testcrosses 1 and 2 shows a total of 13 recombinants in 373 testcross plants, resulting in a pooled recombination value of 3.40 ± 0.24% between Sm_2 and the centromere.

Additional proof that Sm_2 is on chromosome 6 was provided by the observation (Table 2) that Sm_2 was linked with the Lc_2 locus which had already been placed in chromosome 6 (Endrizzi, 1963; Endrizzi and Kohel, 1966). Based on testcross 3, the two markers are separated by 18.86 ± 1.65 map units, which is not significantly different at the 95% confidence limits from the 22.12 map units between Lc_2 and H_2 as reported by Endrizzi and Kohel (1966).

The 3.40 map distance of Sm_2 and the centromere is slightly higher than the map distance between the centromere and the two hairy alleles, H_1 and H_2. In a 2 × 2 homogeneity chi-square analysis of the three sets of data, significant differences occurred only between the two sets involving H_2 and Sm_2 ($\chi^2 = 5.55$, $0.05 < P < 0.01$). The 3.4% recombination (uncorrected) observed between Sm_2 and the centromere is within the limits of sampling variation for the corrected recombination of 4% calculated for the H_2 - centromere distance (Endrizzi and Kohel, 1966). The similarity of these two percentages may be due to no change in recombination in the proximal regions of the telocentric-6L and the standard chromosome carrying the Sm_2 locus. The higher recombination value between Sm_2 and the centromere, however, could be due to the fact that the Sm_2 allele is a recent transfer from an introduced houseyard cotton (Lee, 1968). Only one monotelodisomic-6L F_1 was used for testcrossing. That plant may have had a chromosome with a larger segment of the houseyard strain, that was distal to and included the Sm_2 locus, which tended to

Table 3. Segregation of trichome mutants H_1, H_2 and Sm_2 in F_2 populations of cotton.

Genotypes of crosses†	Number of F_2 plants					
	Hairy	Smooth	Normal	Total	X^2 (3:1)	P
$H_1H_1 \times H_2H_2$	370	0	0	370		
$H_1H_1 \times Sm_2Sm_2$	111	40	0	151	0.179	0.7-0.5
$Sm_2Sm_2 \times H_2H_2$	260	82	0	342	0.191	0.7-0.5

† The lines used with the H_1, H_2 and Sm_2 genes are MU8b, T586, and AG108, respectively.

shift recombination to the proximal region.

In these testcrosses, the telocentric-6L chromosome was transmitted by the pollen ranging from 5.2 (Table 1) to 14.6% (Table 2). The latter frequency is comparable to the 13.1% reported by Endrizzi and Kohel (1966).

Genetic Tests for Allelism

Since the H_1, H_2 and Sm_2 genes appeared to be in the same region of chromosome 6, based on our cytogenetic studies, tests for allelism of the three mutant forms were made by scoring F_2 populations of three intercross combinations (Table 3).

H_1H_2 F_1 plants were very hairy and resembled H_1H_1 plants. The stems and especially the terminal leaves of H_1Sm_2 F_1 plants had trichomes with appearance and density similar to H_1H_1 plants, but the hairs in fully expanded leaves were similar to those of normal plants such as TM-1. The F_1's of Sm_2H_2 were more hairy than normal TM-1 plants, and this was evident by sight and feel of the young terminal leaves.

In the $H_1H_1 \times H_2H_2$ cross, all 370 F_1 individuals were hairy, like the MU8b and T586 lines. This supports the hypothesis that H_1 and H_2 are alleles. Furthermore, there were two types of segregants in the F_2 population. One type was fully hairy like the F_1 and T586 (H_2H_2) and the other was hairy like MU8b (H_1H_1) where the surfaces of older leaves are less hairy. One row consisting of 203 plants was classified into these two types and 146 were fully hairy (H_2H_2 and H_2H_1) and 57 were recorded as typical MU8b. These values give a good fit to the 3:1 ratio with a Chi-square value of 1.03, $P = 0.3$.

In the cross of Sm_2 with H_1 and H_2, the F_2 segregates were either hairy or smooth, and the two types have a good fit to a 3:1 ratio in both crosses (Table 3). In the cross of $Sm_2 \times H_2$, the smooth segregants were indistinguishable from Sm_2Sm_2 plants of the homozygous line AG108. However, in the cross of $Sm_2 \times H_1$, only one F_2 plant was completely smooth as in AG108. All the remaining (39) smooth plants had some plant hairs, but none had the quantitiy of plant hairs typical of that in TM-1, the normal standard. The difference in behavior of the F_2 populations of $H_1 \times Sm_1$ and $Sm_2 \times H_2$ was probably due to difference in genetic background of the two hairy lines. In the development of MU8b, selection was for plants with enhanced hair production to attain maximum Jassid resistance. Similar selection was not performed in the T586 line; consequently expression of the H_2 gene of T586 is not noticeably affected by the genetic background.

In all three F_2 populations, the H_1, H_2, and Sm_2 genes segregated as alleles at a single locus and we believe that the genes are mutants at a single functional unit.

In summary, we believe that the H_1, H_2, and Sm_2 genes are alleles at a common locus in the tetraploid cottons and that a change in the smoothness-hairiness genetic nomenclature is needed.

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