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Duplicate Linkage of Glandless and Nectariless Genes in Upland Cotton, *Gossypium hirsutum* L.[1]

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ABSTRACT

We developed a phenotypic classification system to identify several nectary genotypes in cotton, *Gossypium hirsutum* L. Duplicate linkage groups were established between the glandless genes, gl_2 and gl_3, and the nectariless genes, ne_1 and ne_2. The linkage value for gl_2-ne_1 in linkage group V in the *A* genome was 32.23 ± 1.40% crossover units and the linkage value for gl_3-ne_2 in linkage group IX in the *D* genome was 38.27 ± 1.40% crossover units. The establishment of these linkages marks these two chromosomes so that three-point linkage tests may be conducted to establish the gene order of the duplicate genes for glandless, nectariless, and withering bract.

Additional index words: gossypol, inheritance, pigment, glands.

SMALL lysigenous glands are normally distributed in plants of the genus *Gossypium* in all the aboveground parts. Gossypol is the major chemical constituent of these glands; hence, they have acquired the common name "gossypol glands." Glandless experimental strains of upland cotton have been developed which have virtually no gossypol in the seeds. Research indicates that glandless varieties would make cottonseed a more valuable oilseed for the cotton crushing industry.

Extrafloral and floral nectaries occur in upland cotton, *Gossypium hirsutum* L. (13). Three sets of extrafloral nectaries—the leaf, the outer involucral, and the inner involucral—are prominent in cotton. The floral nectary consists of a ring of papilliform cells at the base of the inner side of the calyx, Tyler (13). Upland lines without extrafloral nectaries have been developed and are termed nectariless. These lines contain only the floral nectary. The nectariless character shows promise as a resistance mechanism of the cotton plant to lepidopterous insect pests (4).

The purpose of this work was twofold: *(1)* to verify a phenotypic classification system for several nectary genotypes, and *(2)* to determine what combination of linkage and independence exists between the glandless genes, gl_2 and gl_3, and the nectariless genes, ne_1 and ne_2.

REVIEW OF LITERATURE

McMichael (5) first produced glandless seed in cotton by combining major genes from 'Hopi Moencopi' with minor genes from upland stocks. He later reported that the glandless character was controlled by the independent recessive genes, gl_2 and gl_3 (6). Lee (2) identified several gland genotypes by gland distribution in the cotyledons. By interspecific crosses he showed that gl_2 and gl_3 are located in the *A* and *D* genomes, respectively (3).

Meyer and Meyer (7) transferred the nectariless trait from *G. tomentosum* N. to *G. hirsutum* L. and reported it to be controlled by the independent recessive genes, ne_1 and ne_2. Rhyne (11), by interspecific hybrids involving diploids and amphidiploids, showed that ne_1 and ne_2 were in the *A* and *D* genomes, respectively. However, he failed to describe all phenotypes for individual genes.

METHODS AND MATERIALS

The original parents used in the study represent four genotypes with regard to glands and nectaries and somewhat variable genetic backgrounds. These are shown in Table 1.

We developed a system of phenotypic classification of nectaries to identify several genotypes in the upland stocks and in progeny produced during this study. These classes are listed with their respective genotypes in Table 2.

Segregation of nectary classes was checked in unison with a linkage study between the glandless and nectariless genes. We used a method developed by Lee (2) to identify the necessary gland genotypes. This method is to observe gland distribution

Table 1. Genotypes and varietal backgrounds of parental cotton lines.*

Genotype	Varietal background and/or phenotypic description
1. $Gl_2\ Gl_2\ Gl_3\ Gl_3\ Ne_1Ne_1\ Ne_2\ Ne_2$	Deltapine Smooth Leaf
2. $gl_2gl_2gl_3gl_3\ ne_1ne_1ne_2ne_2$	M8†, glandless, nectariless
3. $Gl_2\ Gl_2\ Gl_3Gl_3\ ne_1ne_1ne_2ne_2$	M8, nectariless M8, SR-1, nectariless M8, SR-2, nectariless
4. $gl_2gl_2gl_3gl_3\ Ne_1Ne_1Ne_2Ne_2$	M8, glandless

* All lines except Deltapine Smooth Leaf were obtained from Dr. J. R. Meyer, U.S. Department of Agriculture, Agricultural Research Service, Stoneville, Miss.
† M8 originated as a doubled haploid of a Deltapine variety.

Table 2. Phenotypic and genotypic descriptions of the five nectary classes.*

Class	Phenotypic description
A	Full size leaf nectary. Three full size outer involucral nectaries. Three full size inner involucral nectaries. Genotype $Ne_1Ne_1Ne_2Ne_2$ (Figure 1, illustrations 1, 2, 3)†.
D	Full size leaf nectary. Frequently fewer than three outer involucral nectaries and often these are reduced in size. No inner involucral nectaries. Genotypes $Ne_1ne_1Ne_2ne_2$, $Ne_1Ne_1ne_2ne_2$, $ne_1ne_1Ne_2Ne_2$ (Figure 1, illustrations 1, 8, 6).
E	Full size leaf nectary. Outer and inner involucral nectaries absent. Genotype $ne_1ne_1Ne_2ne_2$ (Figure 1, illustrations 1, 5, 6).
F	Reduced leaf nectary--nectary is smaller and may be absent on young leaves. Outer and inner involucral nectaries absent. Genotype $Ne_1ne_1ne_2ne_2$ (Figure 1, illustrations 7, 5, 6).
G	Plants are nectariless. Leaf nectary, outer and inner involucral nectaries absent. Genotype $ne_1ne_1ne_2ne_2$ (Figure 1, illustrations 4, 5, 6).

* The nectary classes were suggested by Dr. H. N. Lafever, formerly of the Boll Weevil Research Laboratory. They were verified by the authors.
† The genotypes $Ne_1ne_1Ne_2Ne_2$ and $Ne_1Ne_1Ne_2ne_2$ were not positively identified in this study. They represent phenotypes similar to A.

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in the cotyledons. We designated the genotypes $Gl_2gl_2Gl_3gl_3$, $Gl_2gl_2gl_3gl_3$, $gl_2gl_2Gl_3gl_3$, and $gl_2gl_2gl_3gl_3$ as gland phenotypes 0, 1, 2, and 3, respectively. This is a modification of Lee's designations. Plants were grown in the greenhouse in peat pots for gland classification. At the three-leaf stage, progeny were transplanted to field plots. Nectary scoring was then conducted when the plants reached the ten-square stage of development.

We collected backcross data to study nectary segregation and gland-nectary linkage. Crosses are described assuming gl_2-ne_1 and gl_3-ne_2 linkage.

Backcross I was a backcross designed to yield repulsion linkage information concerning both gl_2-ne_1, and gl_3-ne_2 linkage groups. Four "families" of this type were produced. "Family" refers to the progeny of a bulked cross. The crosses are shown in Table 3. "Families" 10 and 11 are reciprocal crosses in the F_1 stage.

Table 3. Families in backcross I utilized for repulsion linkage between gl_2-ne_1 and gl_3-ne_2.

"Family" number	Description of cross
10	[(glanded, nectariless × glandless, nectaried) × glandless, nectariless]
11	[(glandless, nectaried × glanded, nectariless) × glandless, nectariless]
12	[(glanded, SR-2 nectariless × M8 glandless nectaried) × glandless, nectariless]
13	[(glanded, SR-1 nectariless × M8 glandless, nectaried) × glandless, nectariless]

Table 4. Phenotypes of heterozygous parental plants in backcrosses II through VII and the genes segregating in each.

Backcross number	Heterozygous parent phenotype	Genes segregating in the backcross population
II	1 F*	gl_2, ne_1
III	2 E	gl_3, ne_2
IV	1 E	gl_2, ne_2
V	2 F	gl_3, ne_1
VI	1 D	gl_2, ne_1, ne_2
VII	2 D	gl_3, ne_1, ne_2

* Gland class 1 and nectary class F.

"Families" 12 and 13 utilize nectariless from two different sources.

We made the cross (glanded, nectariless × glandless, nectaried) and then self pollinated to produce the F_2. In the F_2 we selected several plants for backcrosses II-VII. Individual plants were selected and crossed to glandless nectariless. These crosses were designed to yield segregation of various combinations of the glandless and nectariless genes in the study. Table 4 shows this information.

Backcross VIII was designed to yield coupling linkage data for both gl_2-ne_1, and gl_3-ne_2 linkage groups. The cross was [(Deltapine Smooth Leaf × glandless, nectariless) × glandless, nectariless]. Two "families" were produced.

We made intercrosses of nectary classes E and F for further information on nectary classification. Individual plants for these crosses were selected from a population of [glanded, nectariless × (glanded, nectariless × glandless nectaried)]. Different parents were used to produce each family.

RESULTS AND DISCUSSION

Nectary Classification

Pooled data of four families of backcross I yielded nectary classes D:E:F:G in the ratio of 1:1:1:1 (Table 5). The heterozygous parent plants were phenotype D. These data fit the two gene models for which the classes were designed, indicating that Class D can have the genotype $Ne_1ne_1Ne_2ne_2$ as one of its possible types. Although Chi-square for hetero-

Table 5. Segregation of nectary alleles in several families of five backcrosses.

Backcross no.	Number of families pooled	Nectary classes D	E	F	G	X^2	P	X_h^2	P
I	4	248	240	239	259	1.02	.90-.75	22.61	< .01
III and IV	8		294		285	.12	.75-.50	3.20	.90-.75
II and V	7			225	191	2.76	.10-.05	3.18	.90-.75

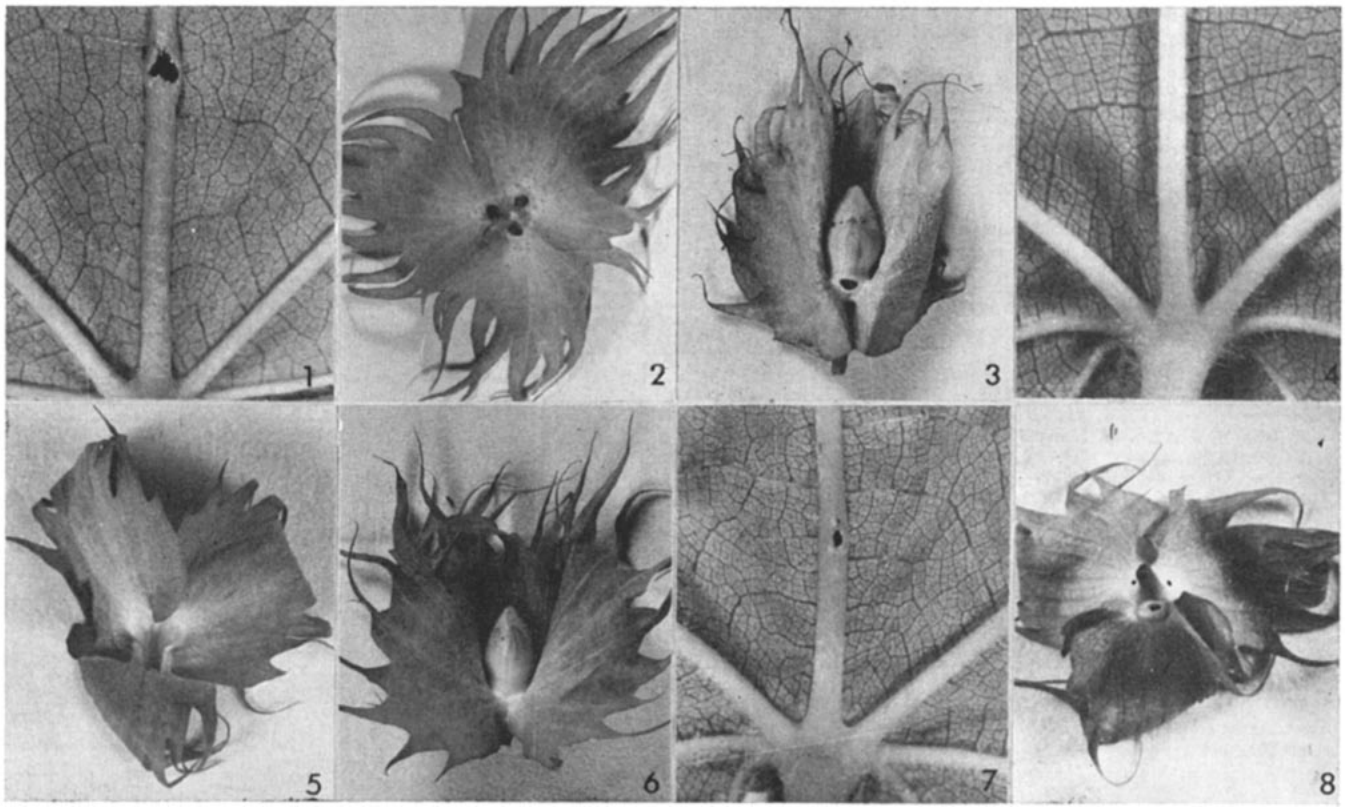

Fig. 1. Variation in nectaries produced by different combinations of the duplicate nectary genes. Nectaries are inked for clarity.

Table 6. Segregation data from backcrosses I through V.

Back-cross no.	Heterozygous parent		No. of families pooled	Gland-nectary phenotype																N	X^2h*	P
	Genotype	Phenotype		0G	1E	2F	3D	0E	1G	2D	3F	0F	1D	2G	3E	0D	1F	2E	3G			
I	Gl_2ne_1 Gl_3ne_2 / gl_2Ne_1 gl_3Ne_2	0D	4	105	100	100	97	64	71	71	57	49	56	52	40	24	33	36	31	986	61.59	.50-.25
IIa	Gl_2ne_1 gl_3ne_2 / gl_2Ne_1 gl_3ne_2	1F	2						15		20						7		6	48	2.24	.90-.75
IIb	Gl_2Ne_1 gl_3ne_2 / gl_2ne_1 gl_3ne_2	1F	1						14		13						30		29	86		
IIIa	gl_2ne_1 Gl_3ne_2 / gl_2ne_1 gl_3Ne_2	2E	2											52	42			37	28	159	5.29	.75-.50
IIIb	gl_2ne_1 Gl_3Ne_2 / gl_2ne_1 gl_3ne_2	2E	1											1	3			8	4	16		
IV	Gl_2ne_1 gl_3Ne_2 / gl_2ne_1 gl_3ne_2	1E	5		103				107						101				94	405	5.61	.97-.95
V	gl_2Ne_1 Gl_3ne_2 / gl_2ne_1 gl_3ne_2	2F	4			84					75			71					52	282	9.84	.50-.25

* X^2 h = Chi square for heterogeneity.

geneity indicated that the four "families" were not homogeneous, the only great discrepancy in the four "families" was in "family" 11. Thirty-seven class E were observed where the expected was 60.

Eight families of backcrosses III and IV yielded essentially equal segregation of classes E and G among 579 progeny (Table 5). Thus, Ne_2-ne_2 segregation was verified and $ne_1ne_1Ne_2ne_2$ was established as the genotype of class E.

Seven families of backcrosses II and V representing 416 progeny produced a 1:1 segregation of F and G classes (Table 5). Thus Ne_1-ne_1 segregation was verified and $Ne_1ne_1ne_2ne_2$ was established as the genotype of class F.

Three types of families were observed in backcrosses VI and VII. Eleven families were scored which produced the E, F, and G phenotypes. Class D was expected in equal frequency with the other three classes. However, class E was observed about twice as frequently as expected. It is suspected that a lack of penetrance of nectary genes resulted in the abnormal segregation. Meyer and Meyer (7) and Rhyne (11) reported a lack of penetrance of nectary genes where plants were under stress. Progeny of backcrosses VI and VII were late-season transplants. Backcross VIII and intercrosses between class E and class F were also late-season transplants and showed reduced penetrance.

Five families of backcrosses VI and VII gave progeny which were all class E. The families contained 22, 45, 63, 116, and 39 plants. The heterozygous parent plants were class D for nectaries. Since the progeny were all class E, the backcross parents all have the genotype $ne_1ne_1Ne_2Ne_2$ which indicates a second possible genotype for the D phenotype.

Two families of backcross VI and VII produced only class F plants. The families consisted of 126 and 128 plants. The heterozygous parent plants were D phenotype for nectaries. Since the progeny were all class F, the genotype $Ne_1Ne_1ne_2ne_2$, was present in the parent plants and is the third genotype possible for the D class phenotype.

Backcross VIII produced 126 D, 161 E, 129 F, and 108 G plants. A 1:1:1:1 ratio was not obtained because of an excess of class E. These progeny were late-season transplants; some reduction in penetrance occurred.

Table 7. Data for gl_2-ne_1 linkage estimates from backcrosses I and II.

Backcross number	NCO	CO	%CO	X^2 linkage
I	665	321	32.55	120.02*
IIa	35	13		10.08*
IIb	59	27		11.91*
(Total for II)	(94)	(40)	(29.85)	
Total	759	361	32.23 ± 1.40	

* Significant for linkage.

Backcross number	NCO	CO	%CO	X^2 linkage
I	599	387	39.24	45.58*
IIIa	94	65		5.29*
IIIb	12	4		4.00*
(Total for III)	(106)	(69)	(39.42)	
Total	705	456	39.27 ± 1.40	

* Significant for linkage.

Table 8. Data for gl_3-ne_2 linkage estimates from backcrosses I and III.

Four families of the intercross class E × class F segregated 2:1:1 for the nectary classes E:F:G. The four families were homogeneous ($X_h^2 = 4.01$, P = .75-.50) and the pooled totals were 43:22:22. These progeny were grown in late season and also showed that a lack of penetrance occurred.

The data show that the nectary phenotypes presented can be used to identify the associated genotypes with a great deal of accuracy. However, variability of classes was shown when plants were under stress.

Linkage Study

Sixteen gland-nectary classes were observed in four families of Backcross I (Table 6). There were four classes of high frequency, eight classes of intermediate frequency, and four classes of low frequency. This distribution obtained indicates the duplicate linkage groups gl_2-ne_1 and gl_3-ne_2. Families were pooled. Alleles at each of the four loci concerned segregated 1:1. The gl_2-ne_1 linkage group exhibited 32.55% crossovers and the gl_3-ne_2 group had 39.24% crossovers (Tables 7 and 8).

Progeny of backcross II segregated into the four expected gland-nectary classes (Table 6). Two families (designated IIa) segregated to indicate gl_2-ne_1 linkage in repulsion phase and one family (designated IIb) segregated to indicate gl_2-ne_1 linkage in coupling phase. Pooled coupling and repulsion data from backcross II show 29.85% crossovers in 134 progeny

for the gl_2ne_1 linkage group, Table 7. Pooled data based on 1,120 progeny from backcrosses I and II showed 32.23 ± 1.40% crossovers between gl_2 and ne_1 (Table 7).

Backcross III produced progeny to verify gl_3-ne_2 linkage (Table 6). Two families (designated IIIa) segregated to indicate repulsion linkage in the backcross parents and one family (designated IIIb) indicated coupling linkage in the backcross parent. A total of 175 progeny of backcross III produced 39.42% crossover in the gl_3-ne_2 linkage group (Table 8). Pooled data based on 1,151 progeny from backcrosses I and III showed 39.27 ± 1.40% crossovers between gl_3 and ne_2 (Table 8).

Five families of the backcross IV segregated 1:1:1:1 for the gland nectary classes 1E, 1G, 3E, and 3G, Table 6. The pooled totals also show independence between gl_2 and ne_2.

A total of 282 plants of backcross V were scored (Table 6). Four families are represented. Each family and the pooled totals showed segregation of the gland-nectary phenotypes 2F, 3F, 2G, and 3G. This segregation showed gl_3 and ne_1 to be independent.

Under nectary segregation we mentioned that backcrosses VI and VII failed to produce the D phenotype. As a result we observed only six of the eight expected gland-nectary classes. Only four of these classes were used to estimate linkage. It was evident that gl_2-ne_1 linkage was present in backcross VI with gl_2 segregating independently of ne_2. Backcross VII showed gl_3-ne_2 linkage and independence of gl_3 and ne_1.

Sixteen gland-nectary classes were observed in backcross VIII similarly as backcross I. However, the classes of high frequency and low frequency reversed, as expected, since backcross VIII was in coupling. The two families were pooled for a total of 524 plants. The data are as follows: OD 55; 1F 40; 2E 43; 3G 52; OF 29; 1D 29; 2G 17; 3E 40; OE 40; 1G 23; 2D 23; 3F 35; OG 16; 1E 38; 2F 25 and 3D 19. Considering the gl_2-ne_1 linkage there were 305 plants in the non-crossover classes and 219 in the crossover classes for a crossing over percentage of 41.79 ± 2.15%. Considering the gl_3-ne_2 linkage there were 311 plants in the non-crossover classes and 213 in the crossover classes for a crossing over percentage of 40.64 ± 2.14%. We think that these crossover values are high for the respective linkage groups. We mentioned previously that an excess of nectary class E occurred. The excess of E phenotypes was noticeable in the crossover classes.

Cotton researchers generally believe that tetraploid *Gossypium hirsutum* L. is an amphidiploid species which originated from a cross between an Old World diploid and a New World diploid *Gossypium* species. The Old World diploid contributed genome *A* and the New World diploid contributed genome *D*.

In an amphidiploid formed by combination of two closely related species one might expect some duplicate characters and duplicate linkage groups. The duplicate linkage groups Yg_2-R_2 and yg_1-R_1 (yellow green and red plant) have been established in both the *A* and *D* genomes, respectively, (18, 9, 12). Kohel et al. (1) and Rhyne (10) reported that the duplicate groups gl_2-bw_1 and gl_3-bw_2 (glandless and withering bract) are in the *A* and *D* genomes, respectively.

This study establishes the linkage groups gl_2-ne_1 and gl_3-ne_2 in upland cotton with 32.23 ± 1.40% and 39.27 ± 1.40% crossovers, respectively, (Tables 7 and 8). Along with these observations the expected independence of gl_2 with ne_2 and gl_3 with ne_1 was shown.

The addition of ne_1 to the gl_2-bw_1 linkage group V and ne_2 to the gl_3-bw_2 linkage group IX, further adds support to the theory that *G. hirsutum* L. originated from a cross between two diploid *Gossypium* species.

The addition of the nectary genes to the estabilshed linkage groups marks the respective chromosomes so that three-point linkage tests may be conducted to establish gene order. The map distance for gl_3-bw_2 is less than five units, but its duplicate gl_2-bw_1 is 12-17 units (1, 11). The addition of the nectariless loci information to each of the linkage groups marks the chromosomes well for a considerable area.

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