THE INHERITANCE OF GOSSYPOL LEVEL IN GOSSYPIUM II:
INHERITANCE OF SEED GOSSYPOL IN TWO STRAINS OF
CULTIVATED *GOSSYPIUM BARBADENSE* L.¹

JOSHUA LEE

U.S. Department of Agriculture, Plant Sciences Division, North Carolina State
University, N.C. 27607

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ABSTRACT

Two strains of cultivated *Gossypium barbadense* L., Sea Island AS-2 and Pima S-4, were used to study the effects of alleles at two loci on the production and/or storage of gossypol in mature embryos. The normal alleles, Gl_2 and Gl_3 , are "native" to *G. barbadense*, whereas the mutant alleles, gl_2 and gl_3 , were introduced from *Gossypium hirsutum* L. through backcrossing. Each strain was grown in three replications per trial, and one, Sea Island AS-2, was grown in three environments. Each experiment consisted of all possible crosses, including reciprocals, of the four true-breeding genotypes, plus parents. Additive effects accounted for more than 90% of the total genetic variance for seed gossypol level in all trials. Epistatic effects, though small, were frequently significant. In *G. barbadense* Gl_2 and Gl_3 were associated with the production of similar amounts of gossypol, whereas previous trials with cultivated varieties of *G. hirsutum* showed that Gl_2 was more than twice as expressive as Gl_3 . The greater average productivity of seed gossypol in cultivated *G. barbadense*, as compared with *G. hirsutum*, was attributed to greater activity at the Gl_3 locus in the former species.

GOSSYPIUM *hirsutum* L. and *Gossypium barbadense* L. are closely related tetraploid species ($4x = 52$) containing wild, ruderal, and intensively cultivated forms (HUTCHINSON, SILOW and STEPHENS 1947; STEPHENS 1950, 1967). Cultivated varieties of *G. barbadense* are known variously as Egyptian, Sea Island, long-stapled cottons, etc., whereas most of the varieties of cultivated *G. hirsutum* are known collectively as upland cottons.

Normally glandular forms of *G. barbadense* and *G. hirsutum* have two major leaf gland loci functional (LEE 1965). The normal alleles at these loci, Gl_2 and Gl_3 , are intimately associated with the production and/or storage of the polyphenolic, gossypol, in mature embryos, whereas the mutant alleles, gl_2 and gl_3 , when homozygous, produce glandless seeds virtually devoid of the substance (McMICHAEL 1960). LEE, COCKERHAM and SMITH (1968), using two varieties of upland cotton, showed that stepwise substitution of normal for mutant alleles

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at the leaf-gland loci resulted in an essentially additive increase in gossypol in mature embryos.

Normally glandular varieties of cultivated *G. barbadense* have produced more gossypol in seeds than cultivated *G. hirsutum* when the two were grown in the same environment (BOATNER *et al.* 1949). Table 1 gives gossypol values for various strains of *G. barbadense* and *G. hirsutum* grown in a uniform trial in a greenhouse at Raleigh during 1972. Thus there is good reason to believe that a random group of *G. barbadense* cottons will produce, on the average, about 0.50 to 0.70% more gossypol in seeds than a similarly selected group of upland cottons.

TABLE 1

Seed gossypol values expressed as percent of dried weight of embryo for some Gossypium barbadense and Gossypium hirsutum cottons, greenhouse 1972

<i>Gossypium barbadense</i>		<i>Gossypium hirsutum</i>	
Variety	Gossypol level	Variety	Gossypol level
Sea Island AS-2	1.913	Coker 100-A	1.456
Sea Island Seaberry	2.146	Carolina Queen	1.443
Sea Island TZRV	2.221	Triple Hybrid 149	1.572
Sea Island Coastland	2.107	Deltapine 15	1.500
Sea Island 12 B ₂	2.472	Acala 4-42	0.967
Amsak	1.961	Acala 1517-D	1.356
Earlipima	1.929	Mu8-b	1.508
Domains Sakel	1.966	Empire	1.274
Pima S-4	2.083	Lankart 57	1.113
Tanguis 5-2/A	1.637	Stoneville 213	1.431
Means	2.043		1.362

In LEE *et al.* (1968) the salient findings were that gossypol level closely paralleled glandulosity of embryos and was largely additive, and that the monomeric genotype $Gl_2Gl_2gl_3gl_3$ produced more than twice as much gossypol as $gl_2gl_2Gl_3Gl_3$. Since *G. hirsutum* and *G. barbadense* are closely related species, it is logical to assume that they produce gossypol through similar pathways. Thus one should expect that gossypol production in seeds of *G. barbadense* should increase in an essentially additive manner when normal alleles are substituted for mutant alleles at the leaf-gland loci. Moreover, the Gl_2 monomeric should produce more than twice as much gossypol as the Gl_3 monomeric, although each should produce proportionately more than its *G. hirsutum* counterpart.

PROCEDURES

In order to test the hypothesis that the gossypol elaborating, or storage, mechanism does not differ in its expression between *G. barbadense* and cultivated *G. hirsutum*, except that it is more potent in the former species, two strains of *G. barbadense* were selected for study. AS-2 Sea Island was selected from the obsolete variety Seabrook Sea Island by S. G. STEPHENS, Department of Genetics, North Carolina State University, to whom I am indebted for supplying the seed of a single inbred plant. Since glandlessness is not known to occur naturally in Sea Island cottons, the character was introduced into the current material through backcrossing with

the upland strain Glandless Empire. Seven backcrosses, attended by careful selection for Sea Island morphological traits, were used in transferring the character. The dimeric, two monomeric, and glandless lines were selected from the segregating generation following the seventh backcross to AS-2. These genotypic lines were then selfed for seed increase.

Pima S-4, the second strain, is a modern American-Egyptian cotton developed by CARL FEASTER of the United State Department of Agriculture, Cotton Research Center, Phoenix, Arizona. I appreciate the help of EDGAR TURCOTTE of the same station for making available both normal and glandless material of this variety. As with AS-2, glandlessness was transferred into the variety through backcrossing with *G. hirsutum*. The two monomerics for Pima S-4 were selected from the F_3 generation following a cross of the glandless and normal lines.

Seeds of the four genotypes were planted in randomized complete blocks with three replications per experiment. There were four plots per genotype, per block, and a minimum of six plants per plot. At flowering, the genotypes were intercrossed or selfed, so that each experiment yielded, at harvest, three replications of a 4×4 diallel set with parents and reciprocal crosses. All pollinations were made within a period of ten days in an attempt to minimize variance due to the possibility that there might be differences in the production of gossypol at different sites on the plant, or during different periods in the season.

After harvest the seed were dried rapidly at 100° F. and stored at sub-freezing temperatures until time was available to process them further. BOATNER *et al.* (1949) showed that seed of *G. barbadense*, when stored at 80° F., increased in gossypol content up to the time the experiment was terminated at 300 days, whereas PONS *et al.* (1948) showed that seed of *G. hirsutum* did not increase in gossypol content if stored at, or below, freezing.

Seed lots were drawn from storage, decorticated, and dried to approximately equilibrium moisture over CaCl_2 (ca. 6%). The kernels were then ground to fine meal and returned to cold storage. After all the seed in a given experiment had been so processed, the samples were extracted and assayed for total gossypol according to the methods of SMITH (1958).

The experiments involving AS-2 were grown in the field during 1970 and 1972, and in the greenhouse during the summer of 1970. Pima S-4 was grown in the field in 1971.

The data were analyzed using methods developed by COCKERHAM (LEE, COCKERHAM and SMITH 1968). There consideration was given to the possibility that there might have been maternal effects, since all heterozygous embryos were produced on mother plants differing reciprocally at one, or both, loci. All gossypol values are given as percent of the total weight of the dried sample of seed meal.

RESULTS AND DISCUSSION

Coefficients of variation for the four experiments, inclusive of field and laboratory error, ranged from 5 to 11%. The proportions of genetic variance assignable to various effects are given in Table 2. More than 90% of the genetic variance in each experiment was attributable to additive effects, a finding similar to that

TABLE 2
Proportions of genetic variance assignable to various classes

	AS-2, field 1972		AS-2, field 1970		AS-2, greenhouse 1970		Pima S-4, field 1971	
	Variance	Percent	Variance	Percent	Variance	Percent	Variance	Percent
Additive	0.439	95	0.305	92	0.416	96	0.307	96
Dominance	0.002	0	0.000	0	0.001	0	0.002	0
Epistatic	0.022	5	0.025	8	0.017	4	0.011	4
		100		100		100		100

TABLE 3

Mean gossypol yields by genotype for AS-2, field 1972 (1), field 1970 (2), greenhouse 1970 (3), and Pima S-4, field 1971 (4)

Female	Male			
	$Gl_2Gl_2Gl_3Gl_3$	$Gl_2Gl_2gl_3gl_3$	$gl_2gl_2Gl_3Gl_3$	$gl_2gl_2gl_3gl_3$
$Gl_2Gl_2Gl_3Gl_3$ (1)	2.130	1.888	1.978	1.268
(2)	1.735	1.429	1.589	1.007
(3)	2.188	1.459	1.792	0.976
(4)	1.972	1.213	1.248	0.760
$Gl_2Gl_2gl_3gl_3$ (1)	1.852	1.171	1.412	0.320
(2)	1.443	1.021	0.981	0.110
(3)	1.410	0.871	0.984	0.068
(4)	1.351	0.743	0.708	0.135
$gl_2gl_2Gl_3Gl_3$ (1)	1.976	1.265	1.401	0.421
(2)	1.540	1.028	1.176	0.213
(3)	1.693	1.013	1.113	0.195
(4)	1.364	0.735	0.763	0.017
$gl_2gl_2gl_3gl_3$ (1)	1.280	0.253	0.358	0.014
(2)	1.009	0.109	0.210	0.010
(3)	0.970	0.064	0.196	0.007
(4)	0.770	0.080	0.008	0.004
Grand means: (1) 1.187	(3) 0.941			
(2) 0.913	(4) 0.742			

made earlier with *G. hirsutum* (LEE, COCKERHAM and SMITH 1968). There were small, though frequently significant, estimates of epistatic variance, and no dominance or maternal effects.

As for average yields of gossypol per genotype (Table 3), *G. barbadense* differed from *G. hirsutum* in two respects: (1) most of the genotypes producing glands produce more gossypol than their counterparts in *G. hirsutum* (2) the Gl_3 monomeric in *G. barbadense* proved to be as expressive in *G. barbadense* as the Gl_2 monomeric. In fact, in AS-2 the Gl_3 monomeric yielded, on the average, more gossypol than the Gl_2 monomeric ($t_{16} = 3.18^{**}$).

Although the monomerics for Pima S-4 and AS-2 differed somewhat in gossypol level, the dimeric genotype was about equally productive in both. One of the dimeric values for AS-2 was somewhat lower than the other two. This low value may have been caused by dry conditions during late summer in 1970. PONS, HOFFPAUIR and HOPPER (1953) showed a positive correlation between rainfall during the period of seed maturation and seed gossypol level in several varieties of *G. hirsutum*. It is likely that *G. barbadense* responds in a similar way to moisture stress or abundance.

Seemingly, the major difference in seed gossypol production and/or storage in normally glandular varieties of *G. hirsutum* and *G. barbadense* is conditioned by the greater productivity of the Gl_3 allele in the latter species. This conclusion is supported by the following. *Gossypium raimondii* Ulb. is a diploid species conceded to be one of the putative ancestors of the tetraploid species of *Gossypium* (HUTCHINSON, SILOW and STEPHENS 1947). It has a single leaf-gland locus ac-

tive (LEE 1965), and the allele at this locus is the homolog of Gl_3 in tetraploids. After this allele was combined with Gl_2 in the upland variety Empire, I recovered a dimeric line of the genotype $Gl_2Gl_2Gl_3^{rai}Gl_3^{rai}$ which averaged 1.913% gossypol in mature embryos. Thus the incorporation of a more potent allele from the putative diploid parent raised an ordinary upland (ca. 1.20 to 1.30% gossypol) to a level similar to that of some cultivated strains of *G. barbadense*.

According to FRAMPTON, PONS and KERR (1960) and CARTER *et al.* (1966) *G. raimondii* produces seed gossypol in the range of 2.54 to 3.68%. Thus the potential productivity of Gl_3^{rai} drops drastically when it is introgressed into *G. hirsutum*. Several values for monomerics involving this allele in Empire background have ranged between 0.700% and 0.800% (WILSON and LEE 1971).

At present there is little information on the pathways for the production and storage of gossypol in the cotton plant, so there is no way of knowing why Gl_3^{rai} drops in expressivity when it is introgressed into *G. hirsutum*. However, the fact that it seemingly stabilizes at a point near the productivities of both Gl_2 and Gl_3 in *G. barbadense*, and Gl_2 in *G. hirsutum*, suggests that these latter alleles have the potential for wild-type expression should they be transferred to the proper background, a background such as *G. raimondii*, or to *Gossypium herbaceum* var. *africanum* (Watt) Hutchinson et Ghose. Seeds of the latter species recently assayed 3.16% gossypol. *Gossypium herbaceum* is also a putative ancestor of the cultivated tetraploid cottons (GERSTEL 1953), having apparently supplied the A genome, and thus the Gl_2 allele (LEE 1965).

If these species, or similar forms, were, indeed, the ancestors of the tetraploid cottons, the primitive tetraploids must have had much higher seed gossypol potentials than modern-day cultivated forms. This speculation seems to be borne out by the fact that wild and ruderal forms of both *G. hirsutum* and *G. barbadense* have recently assayed as high as 4.00% gossypol in seeds from material grown in a greenhouse at Raleigh. Moreover, there must have been a considerable amount of redundancy in potential function at the leaf gland loci in primitive tetraploids. HUTCHINSON, SILOW and STEPHENS (1947) theorized that such redundancy would be reduced over time through deterioration in the parts of the genetic mechanism shielded from selection. If this were the case, cultivated *G. hirsutum* and *G. barbadense* have evolved to the point where there is little apparent redundancy in the system that produces the pigment glands and, concomitantly, produces and/or stores gossypol, since in both the process is virtually linear over the two loci in question.

One should be able to interchange Gl_2 between the two species without any disturbance in the level of gossypol production in either. However, exchange of Gl_3 should result in lower gossypol production in the seeds of *G. barbadense* accompanied by raised production in *G. hirsutum*. Material is being prepared to test this hypothesis.

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Corresponding Editor: R. ALLARD