THE INHERITANCE OF GOSSYPOL LEVEL IN GOSSYPIUM I.
ADDITIVE, DOMINANCE, EPISTATIC, AND MATERNAL EFFECTS
ASSOCIATED WITH SEED GOSSYPOL IN TWO
VARIETIES OF GOSSYPIUM HIRSUTUM L.¹

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Studies in quantitative inheritance are generally conducted as the analysis
of the effects of groups of genes acting in concert to produce the character
under consideration. Rarely can the number of loci involved be known. It is thus
of some interest when the number of genes involved in the production of a char-
acter can be known through the use of a method which allows for discrimination
among units of expression by qualitative assays, yet have the genes express them-
theselves in some other way, the nature of which is quantitative. The character,
expression of pigment gland size and number in the plant body and seeds of
various species of Gossypium, fits into this category.

A normally glandular plant of Upland cotton (Gossypium hirsutum var.
latifolium Murray) displays pigment glands in the leaves, stems and carpel walls
in undiminishing numbers throughout the lifespan of the plant. When such a
plant is crossed to a plant devoid of glands, the resulting F₁ is approximately
intermediate in glandulosity. The F₂ generation displays a continuous array of
glandulosity ranging from a very few glands about the margins of the cotyledons
to types which appear to be normal. In addition there are a few glandless indi-
viduals. McMichael (1960) compared the F₂ progenies stemming from the
cross of a glandless strain to several accessions of Upland cotton. He concluded
from the relative numbers of glandless seedlings in each progeny that the con-
certed action of alleles at two independent loci produced the glandless character.
Lee (1962) devised a method for separating the active, gland-producing loci
through inspection of the pattern of glands on the cotyledons of week-old seed-
lings. The two monomeric genotypes produced in this fashion proved to be
strikingly different in their relative abilities to sustain gland production through
various phases of plant development.

Pigment glands in cotton contain a variety of polyphenolic substances (Stan-

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FORD and VIEHOVER 1918). One of these, gossypol, occurs in the seed practically to the exclusion of any of the others. Thus an assay of the gossypol of the seed of a given strain gives a close estimate of the total polyphenol stored in a given amount of embryonic tissue and should correlate well with the size and number of glands in the sample. Gossypol analysis of the monomeric stocks obtained from partitioning the active loci of a commercial cotton commonly grown in the southeastern United States revealed that one monomeric produced about twice as much of the substance as the other, as measured by percent of the dried weight of the embryonic tissue which was gossypol. Moreover, the gossypol level of the seed related closely with the overall expression of glandulosity of individual plants of a particular genotype. Selfed seeds from glandless plants had a very low level of gossypol.

Since gossypol level can be measured easily, and with a great deal of precision, and genotypes of known constitution produced at will, it was thought worthwhile to produce all of the possible genotypes and analyze the results to detect the mode of gene action in this two-locus system.

EXPERIMENTAL PROCEDURES

Two cotton varieties, Empire 61 (WR) and Coker 100-A, both normally glandular cottons of the genotype $GL_2GL_2GL_3GL_3$, were backcrossed repeatedly to a glandless donor strain of the genotype $gl_2gl_2gl_3gl_3$. After 5 generations of backcrossing, appropriate selection techniques (LEE 1962) were used to secure plants of the four possible, true-breeding, genotypes within each variety. About 12 plants per genotype were selected. These were selfed in order to secure a seed increase. Moderate selection for recurrent varietal characteristics was practiced during the backcrossing program in order to hasten the production of near-isogenic lines, resembling, as closely as possible in all other respects, the recipient lines.

The four genetic lines within each variety were planted in the field in randomized complete blocks with 3 replications per entry. A uniformity test, performed earlier to compare the relative precision of field vs. laboratory sampling, revealed that with the level of precision expected in the laboratory, 3 replications in the field would allow for the detection of a difference of 0.20% in gossypol level between 80 and 90% of the time at the 95% level of significance. Each plot consisted of 8 hills of 3 plants each. Each cross and its reciprocal was allotted one plot per replication. Parental material was taken from selfing a single plot in each replication. The crosses, reciprocals and parental material obtained from the experiment constituted a $4 \times 4$ diallel set with reciprocals for each variety.

All crosses and self-pollinations were made within a period of 10 days. This limiting of the pollination period represented an attempt to minimize errors induced by environmental causes such as relative maturity of embryos as related to position on the plant. About one dozen fruit per plot were considered an adequate sample for the purposes of the experiment.

The seeds, after harvest, were acid-delinted and subjected to flotation in water in order to remove seeds of low specific gravity, since these seeds generally contain immature embryos in which the gland complement is not fully developed.

A random sample of 50 seeds was taken from the remaining seeds from each plot. These seeds were decorticated and assayed for gossypol level according to the methods of SMITH (1958). Each plot datum was taken as the mean value of two laboratory determinations per sample of seed and expressed as percent of gossypol in the dried embryonic tissue.

3 Regional Cotton Genetics Project S-1 for the Calendar year 1964; pages 11–14.
Since the cross seed in each case were harvested from the maternal parent there was some concern that the maternal and paternal (only gametic) contributions were different. Also, since the factorial and diallel are standard forms of analyses (Table 1), it seemed worthwhile to relate these to each other and to individual degrees of freedom comparisons corresponding to various genetic and maternal effects. The sums of squares and degrees of freedom for the diallel correspond to those of Hayman (1954) without further subdivision of his b sum of squares. First, models corresponding to each analysis will be developed and related to the maternal and genetic model.

Let the mean of a cross be represented as:

$$\bar{Y}_{ii'jj'} = \mu + g_{ii'}^m + g_{jj'}^p + (gg)^{ii'}_{jj'} + \epsilon$$

where $i,j'$ index the genes from the maternal parent and $j,j'$ those from the paternal parent. Further, $i$ and $j$ index the $Gl_2$ genes,

- $i$ or $j = 0$ for $Gl_2$
- $i$ or $j = 1$ for $gl_2$,

and, $i'$ and $j'$ index the $Gl_3$ genes:

- $i'$ or $j' = 0$ for $Gl_3$
- $i'$ or $j' = 1$ for $gl_3$.

When needed to avoid confusion we shall also use $a_i$ or $a_j$ for $Gl_2$ genes and $b_{i'}$ or $b_{j'}$ for $Gl_3$ genes.

For the factorial model we have:

$$\bar{Y}_{ii'jj'} = \mu + g_{ii'}^m + g_{jj'}^p + (gg)^{ii'}_{jj'} + \epsilon$$

**TABLE 1**

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factorial</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replications</td>
<td>2</td>
<td>$S_r$</td>
</tr>
<tr>
<td>Male—$g^p$</td>
<td>3</td>
<td>$S_{p}^p$</td>
</tr>
<tr>
<td>Female—$g^m$</td>
<td>3</td>
<td>$S_{p}^m$</td>
</tr>
<tr>
<td>MxF—$(gg)^{mp}$</td>
<td>9</td>
<td>$S_{(gg)}^{mp}$</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>$S_e$</td>
</tr>
<tr>
<td><strong>Diallel</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replications</td>
<td>2</td>
<td>$S_r$</td>
</tr>
<tr>
<td>General—$G$</td>
<td>3</td>
<td>$S_G$</td>
</tr>
<tr>
<td>Specific—$(GG)$</td>
<td>6</td>
<td>$S_{(GG)}$</td>
</tr>
<tr>
<td>Maternal—$M$</td>
<td>3</td>
<td>$S_m$</td>
</tr>
<tr>
<td>Reciprocal—$(MM)$</td>
<td>3</td>
<td>$S_{(MM)}$</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>$S_e$</td>
</tr>
</tbody>
</table>
\( \mu \) is the population mean,
\( g^m \) is the effect of the maternal parent,
\( g^p \) is the effect of the parental parent,

\[(gg)^{mp} \] is the interaction of the two,
\( \epsilon \) is the environmental error corresponding to the mean of the observations.

There is every reason to assume that the paternal gametic effect, \( g^p \), is entirely genetic and expressed only through genes in the embryo. Consequently, we can express this gametic effect in terms of effects for the genes in the gametes

\[ g^p_{ij'} = a_{ij} + a_{ij'} + (\alpha \alpha)_{ij'} \]  

(2)

where:
\( a \) is the average or additive effect of the gene,
\( (\alpha \alpha) \) is the first order or additive by additive interaction of the two nonalleles.

Maternal effects in this case may be a direct result of the genotype of the maternal parent or gametic other than the genes under consideration. In either case, or both, they are incorporated into \( m_{ii'} \) and:

\[ g^m_{ii'} = m^m_{ii'} + g^p_{ii'} + (mg)^{mp}_{ii'} \]  

(3)

where:
\( g^p = g^m \) of paternal parent,
\( m^m \) is the maternal effect,
\( (mg)^{mp} \) is the interaction of the two.

As a direct effect of the genotype of the parent,

\[ m^m_{ii'} = \theta_{ii} + \theta_{ii'} + (\theta \theta)_{ii'} \]  

(4)

where:
\( \theta \) is the average direct effect of the two homozygous alleles in homozygous parents on their seed,
\( (\theta \theta) \) is the interaction effect for the two loci.

The expansion of \( (mg)^{mp}_{ii'} \) into interaction effects is straightforward, being composed of the interactions of the elements of \( m^m_{ii'} \) in (4) with the elements of \( g^p_{ii'} \) in (2).

Returning to the original model

\[(gg)^{mp}_{ii'} = (mg)^{mp}_{ii'} + (gg)^{mp}_{ij'} + (mgg)^{mp}_{ii'} \]  

(5)

The expansion of \( (mg)^{mp}_{ii'} \) in terms of embryonic and maternal gene effects is the same as for \( (mg)^{mp}_{ii'} \) except of course effects corresponding to certain combinations of genes never occur in the latter term. The embryonic gene effects are of primary concern and the gene interactions between gametes are:

\[ (gg)^{p}_{ij'} = \delta_{a_{i}a_{j}} + \delta_{b_{i}b_{j}} + (\alpha/\alpha)_{a_{i}b_{j}} + (\alpha/\alpha)_{b_{i}a_{j}} \]

+ \( (\alpha \delta)_{a_{i}b_{i},a_{j}} + (\alpha \delta)_{a_{i}b_{i},b_{j}} \)

+ \( (\delta \delta)_{a_{i}b_{i},a_{j}} \)  

(6)
where δ stands for dominance and α for additive in the nomenclature of the effects. The notation \((a/α)\) is used to distinguish the additive by additive interaction of genes from different gametes as opposed to \((aa)\) for genes in the same gamete. For them to be different requires a cis-trans effect, called configuration effect by Griffing (1961), or in simplest terms, a difference between double heterozygotes. While such a distinction could apply only to genes on the same chromosome, it seemed worthwhile to illustrate the estimation of the two separately.

The diallel analysis (Table 1) for which the partitions of the sums of squares are additive (i.e., add to the total, and are orthogonal in the errors, \(e's\), to each other) results from the following model (Hayman 1954):

\[
\begin{align*}
\bar{Y}_{i'j'j'} &= \mu + G_{i'j'} + G_{jj'} + M_{ii'} - M_{jj'} + (GG)_{i'j'j'} \\
&\quad + (MM)_{i'j'j'} + \bar{e}
\end{align*}
\]

where:

- \(G\) is the general effect,
- \(M\) is the maternal vs. paternal effect,
- \((GG)\) is the specific effect,
- \((MM)\) is the reciprocal effect,
- \((GG)_{i'j'j'} = (GG)_{jj'i'}\),
- \((MM)_{i'j'j'} = -(MM)_{jj'i'}\).

The effects are related to those of the factorial model (1) in the following ways:

\[
\begin{align*}
G_{i'j'} &= (g_{i'j'} + g_{j'i'}/2 \\
M_{i'j'} &= (g_{i'j'} + g_{j'i'}/2
\end{align*}
\]

\[
\begin{align*}
(GG)_{i'j'j'} &= [(gg)_{i'j'j'} + (gg)_{j'i'i'}]/2 \\
(MM)_{i'j'j'} &= [(gg)_{i'j'j'} - (gg)_{j'i'i'}/2
\end{align*}
\]

Expansion of \(g_{i'i'}\) as in (3) leads to:

\[
\begin{align*}
G_{i'i'} &= g_{i'i'} + [m_{i'i'} + (mg)_{i'i'}/2 \\
M_{i'i'} &= [m_{i'i'} + (mg)_{i'i'}/2
\end{align*}
\]

Note that \(M_{i'i'}\) involves only direct maternal effects and interaction of maternal with gametic genetic effects, while \(G_{i'i'}\) contains, in addition, gametic genetic effects expressed by the embryo. Proceeding in a similar manner for the interaction effects:

\[
\begin{align*}
(MM)_{i'i'j'} &= f_1(\theta, αδ) \\
(GG)_{i'i'j'} &= (gg)_{i'i'j'} + f_2(\theta, αδ) + f_1(θ, αδ)
\end{align*}
\]

since:
and where \( f_1 (\theta, \alpha \delta) \) and \( f_2 (\theta, \alpha \delta) \) are different functions of interactions involving \( \theta \)'s with \( \alpha \)'s and \( \delta \)'s. Again, note that the reciprocal effects, \((MM)\)'s, involve only maternal by embryonic gene interactions, while the specific effects, \((GG)\)'s, involve additional ones plus interactions among gametic genes.

One should pause at this point to reflect on the purpose of the analysis. It is to estimate and test hypotheses about as many gene effects as possible, and, in particular, to make some separation of embryonic and maternal gene effects. On the surface it appears that some separation is possible, but this turns out not to be always the case. The surface impression depends on the often held misconception that when main effects are estimated by forcing interactions to add to zero in convenient ways, the estimates are unaffected or “free” of these interaction effects. Thus, one might decide that even if a test of reciprocal effects indicated maternal effects to be important, the factorial analysis could be used to obtain unbiased estimates of the \( g^e \)’s and thus of the \( \alpha \)'s and \( (\alpha \alpha) \). Actually, as is often overlooked, the restrictions imposed on interactions very much determine what main effects estimate.

There are of course many more effects defined in the model than can be discerned from the data. What is estimated by the factorial and diallel analyses can be illustrated by first expressing each cross in terms of the effects, which is done in Table 2 for all embryonic main and interaction gene effects and for maternal gene effects but not for interactions of embryonic and maternal gene effects.

### TABLE 2

*Embryonic and maternal gene effects for each genotype*

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>( a_a )</th>
<th>( a_b )</th>
<th>( (\alpha \alpha)_{ab} )</th>
<th>( \delta_a )</th>
<th>( \delta_b )</th>
<th>( (\alpha / \alpha)_{ab} )</th>
<th>( (\alpha \delta)_{ab} )</th>
<th>( (\alpha \delta)_{ba} )</th>
<th>( (\delta \delta)_{ab} )</th>
<th>( \theta_a )</th>
<th>( \theta_b )</th>
<th>( (\theta \theta)_{ab} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a_i b_i / a_j b_j )</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( a_i b_i / a_j b_k )</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( a_i b_i / a_j b_0 )</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( a_i b_i / a_j b_1 )</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( a_i b_i / a_j b_2 )</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( a_i b_i / a_j b_3 )</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( a_i b_i / a_j b_4 )</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( a_i b_i / a_j b_5 )</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Genotype = female gamete/male gamete*
Restrictions have already been imposed that all effects of a class sum to zero over all indexes. For example:

\[
\begin{align*}
\alpha_a &= \alpha_{a_0} = -\alpha_{a_1} \\
(\alpha\alpha)_{ab} &= (\alpha\alpha)_{a_0b_0} = - (\alpha\alpha)_{a_1b_1} = - (\alpha\alpha)_{a_2b_2} = (\alpha\alpha)_{a_3b_3} \\
\delta_a &= \delta_{a_0} = - \delta_{a_1} = \delta_{a_2} \\
(\alpha\delta)_{ab} &= (\alpha\delta)_{a_0b_0} = - (\alpha\delta)_{a_1b_1} = - (\alpha\delta)_{a_2b_2} = (\alpha\delta)_{a_3b_3} \\
\theta_a &= \theta_{a_0} = - \theta_{a_1} \\
\end{align*}
\]

and so on.

The restrictions for embryonic gene effects were imposed systematically without regard to maternal or paternal source. To complete the expression of effects for each genotype in Table 1 to include interactions of maternal with embryonic effects, one multiplies the coefficients of the nine embryonic effects by the coefficients of the three maternal effects leading to the coefficients of the 27 interaction effects ranging from \((\alpha\theta)_{ab}\) to \((\delta\theta\theta)_{abab}\).

Individual degree of freedom comparisons are given in Table 3 for the diallel analysis and in Table 4 for main effects of the factorial analysis. One orthogonal breakdown of the interaction effects for the factorial analysis corresponds to those comparisons in specific and reciprocal of the diallel analysis since \(S(GG)_{mp} = S(\theta\theta)_{mp} + S(MM)\) (Table 1). Also, \(S_{pp} + S_{pm} = S_G + S_M\), the total being partitioned in different ways by the two analyses. The comparisons in Tables 3 and 4 are identified by the principal low order effects in the comparison and are starred to indicate that other effects are involved. There are many aliases of effects because the model exceeds the power of the experiment to discriminate, mainly because of the confounding of the maternal genotype with the maternal gamete. For example, the comparison labeled \((\delta\theta\theta)_{abab}\) in Table 3 could be found operationally in other ways,

\[
(\delta\theta\theta)_{abab} = -(\delta\theta\theta)_{baba} = (\alpha\theta)_{ab} - (\alpha\theta)_{ab} .
\]

The effects involved in each comparison are found by performing the comparison for each column of effects in Table 2, including the extension of the table to include interactions of embryonic and maternal gene effects. At the bottom of Table 3 are given alternate comparisons to \((\alpha\alpha)_{ab}\) and \((\alpha/\alpha)_{ab}\),

\[
(\alpha\alpha)_{ab} = \frac{(\alpha\alpha)_{ab} + (\alpha/\alpha)_{ab}}{2}
\]

\[
C* = \frac{(\alpha\alpha)_{ab} - (\alpha/\alpha)_{ab}}{2}.
\]
TABLE 3

Individual degree of freedom comparisons for the diallel analysis

| Genotype* | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | $a_{\phi_0}$ | $a_{\phi_1}$ | Divisor |
|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| General   |             |             |             |             |             |             |             |             |             |             |             |             |             |             |               |             |               |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
| $\alpha_a*$| 1           | 1           | .           | .           | 1           | 1           | .           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | 16         |
| $\alpha_b*$| 1           | .           | 1           | .           | .           | 1           | .           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | 16         |
| $(\alpha \alpha)_{ab}$*| 1           | .           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | 16         |
| Specific  |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |               |             |               |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
| $\delta_a*$| 1           | 1           | .           | .           | 1           | 1           | .           | .           | 1           | 1           | .           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | 16         |
| $\delta_b*$ | 1           | .           | 1           | .           | 1           | 1           | .           | .           | 1           | 1           | .           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | 16         |
| $(\alpha / \alpha)_{ab}$* | 1           | .           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | 16         |
| $(\alpha \delta)_{ab}$* | 1           | .           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | 16         |
| $(\delta \delta)_{ab}$* | 1           | .           | .           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | .           | 1           | .           | .           | 1           | .           | 1           | .           | 1           | .           | 1           | .           | 16         |
| Maternal  |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |               |             |               |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
| $\theta_a*$ | .           | .           | 1           | 1           | .           | 1           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | 8          |
| $\theta_b*$ | .           | .           | .           | 1           | .           | .           | .           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | 8          |
| $(\theta \theta)_{ab}$* | .           | .           | .           | 1           | .           | .           | .           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | 8          |
| Reciprocal |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |               |             |               |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
| $(\delta \theta)_{ab}$* | .           | .           | .           | .           | 1           | .           | .           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | 8          |
| $(\delta \theta)_{ba}$* | .           | .           | .           | .           | 1           | .           | .           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | 8          |
| $(\delta \delta \theta)_{ab}$* | 1           | .           | .           | .           | 1           | .           | .           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | 8          |
| $(\alpha \alpha \theta)_{ab}$* | .           | .           | .           | .           | 1           | .           | .           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | .           | 1           | 1           | 8          |

* Genotype =
  female gamete
  male gamete
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Individuals</th>
<th>Parental</th>
<th>( a_0 )</th>
<th>( a_1 )</th>
<th>( a_{10} )</th>
<th>( a_{11} )</th>
<th>( a_{101} )</th>
<th>( a_{100} )</th>
<th>Divisor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td>( a_{00} )</td>
<td>( a_{11} )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( a_{01} )</td>
<td>( a_{10} )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( a_{000} )</td>
<td>( a_{111} )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( a_{001} )</td>
<td>( a_{101} )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( a_{010} )</td>
<td>( a_{110} )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( a_{011} )</td>
<td>( a_{100} )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( a_{100} )</td>
<td>( a_{0} )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( a_{101} )</td>
<td>( \theta )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( a_{110} )</td>
<td>( \beta )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>( a_{111} )</td>
<td>( \gamma )</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 4**: Individual degree of freedom comparisons for the main effects of the factorial analysis.
sponds to Griffing's (1961) configuration effect. The divisors in Tables 3 and 4 are the ones which lead to coefficients of one for the principal effect under consideration, and when the comparisons are of means for the genotypes. When the comparison is of sums, the divisor must be multiplied by the number of observations (replicates) on a genotype.

Pertinent comparisons will be given, the full result being straightforward but tedious. Also, comparable comparisons, for example \( a^*b \) from \( a^*a \), can be found by interchanging \( a \) and \( b \). We know from (10) that reciprocal effects involved only interactions of embryonic and maternal gene effects. These are of the type

\[
(\delta \theta)_{ab} = (\delta \theta)_{ab} + (a\delta \theta)_{aab} - (a\delta \theta)_{abb} + (a\alpha \delta \theta)_{aab} - (a\alpha \delta \theta)_{abb} + (a\alpha \theta)_{a} - (a\alpha \theta)_{b}
\]

(14)

We also know from (9) that maternal effects included interactions of maternal and embryonic effects:

\[
(\theta \alpha)_{ab} = (\theta \alpha)_{ab} + (\alpha \theta)_{ba} + (a\theta)_{ab} - (a\delta \theta)_{baa} - (a\delta \theta)_{bab} + (a\delta \theta)_{bba} + (a\delta \theta)_{bab}
\]

(15)

The results for general effects are:

\[
(\alpha^*a)_{a} = \alpha_{a} + \theta_{a} + (\delta \theta)_{aa} + (a\alpha \theta)_{aab} + (a\alpha \theta)_{abb} + (a\alpha \theta)_{b} + (a\alpha \theta)_{b}
\]

(16)

and for specific effects are:

\[
(\alpha^*a)_{ab} = (\alpha^*a)_{ab} + [(\delta \theta)_{ba} + (a\alpha \theta)_{bab} + (a\alpha \theta)_{bab}] / 2
\]

(17)

If there are no configuration effects, similar types of effects involving \( \alpha \alpha \) and \( \alpha \alpha \) are the same, e.g., \( (\alpha \alpha)_{ab} = (\alpha \alpha)_{ab} \), \( (a\alpha \theta)_{abb} = (a\alpha \theta)_{abb} \), etc.

The comparisons for the factorial analyses are found directly from:

\[
\alpha^{*a} = \alpha^{*a} + \theta^{*a} / 2
\]

\[
\alpha^{*a}, \theta^{*a} = \alpha^{*a} + \theta^{*a} / 2
\]

(18)
INHERITANCE OF GOSSYPOL LEVEL

\[(aa)_{ab} = (aa)_{ab} - (\theta \theta)_{ab}/2\]

\[(aa)_{ab} + (\theta \theta)_{ab} = (aa)_{ab} + (\theta \theta)_{ab}/2.\]

These results, (14) through (18), clarify the source of estimates, and state which hypotheses are testable in the analyses. A test of reciprocal effects is a test of interactions of maternal with embryonic effects. The test is of a conglomerate, however, and nonsignificance does not guarantee that other effects, general, maternal or specific, are not affected by this interaction, since different functions are involved. Only under the assumption of no gene interactions of maternal with embryonic can the maternal or embryonic gene effects be estimated unbiasedly. While again involving conglomerates of effects, nonsignificant maternal effects in conjunction with nonsignificant reciprocal effects would certainly provide confidence in the unbiasedness of estimates of embryonic gene effects from general and specific effects.

If there are maternal gene effects but no interactions with embryonic gene effects, then the \(\theta\)’s and \((\theta \theta)\) are estimated from maternal and the \(\alpha\)’s and \((aa)\) from the male effects of the factorial analysis. When there are no maternal effects, all of the information about the \(\alpha\)’s is combined into the general effects which provide the best estimates.

RESULTS

The values in Table 5 are sums of three plots for each of the background varieties. Parental data in Table 5 were taken from selfed seed.

An orthogonal partitioning of the variation corresponding to that in Table 3 is given in Table 6. The results for the two varieties are very similar, and in neither were maternal or reciprocal effects, whether tested individually or as composites, indicated to be important. Consequently, one can proceed with some confidence in testing and estimating the embryonic gene effects. All were significant except for the additive by additive ones.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a_0b_0)</td>
<td>(a_0b_1)</td>
<td>(a_1b_0)</td>
<td>(a_1b_1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.109</td>
<td>3.242</td>
<td>2.222</td>
</tr>
<tr>
<td></td>
<td>(3.756)</td>
<td>(3.312)</td>
<td>(2.624)</td>
<td>(1.793)</td>
</tr>
<tr>
<td></td>
<td>3.863</td>
<td>2.810</td>
<td>2.110</td>
<td>0.470</td>
</tr>
<tr>
<td></td>
<td>(3.689)</td>
<td>(2.545)</td>
<td>(1.893)</td>
<td>(0.291)</td>
</tr>
<tr>
<td></td>
<td>2.834*</td>
<td>1.975</td>
<td>1.211</td>
<td>0.140</td>
</tr>
<tr>
<td></td>
<td>(2.673)</td>
<td>(1.890)</td>
<td>(0.995)</td>
<td>(0.162)</td>
</tr>
<tr>
<td>Female</td>
<td>2.115</td>
<td>0.354</td>
<td>0.123</td>
<td>0.064*</td>
</tr>
<tr>
<td></td>
<td>(1.853)</td>
<td>(0.250)</td>
<td>(0.124)</td>
<td>(0.035)</td>
</tr>
<tr>
<td></td>
<td>(11.980)</td>
<td>(7.997)</td>
<td>(5.636)</td>
<td>(2.281)</td>
</tr>
</tbody>
</table>

Upper values are Coker 100-A, lower values (in parentheses) are Empire 61 (WR)
The mean effects were found by operating on the sums as given in Table 3 with the divisor increased by a factor of three for the three replications.

The variances are estimates of those for a noninbred linkage equilibrium population with gene frequencies of one half. In a non-inbred linkage equilibrium population the total genetic variance is

$$\sigma^2_h = 2\sigma^2_a + 2\sigma^2_b + \sigma^2_{\delta a} + \sigma^2_{\delta b} + 2\sigma^2_{(aa)_{ab}} + 2\sigma^2_{(a/\delta)_{ab}} + 2\sigma^2_{(a\delta)_{ba}} + \sigma^2_{(\delta\delta)_{ab}},$$

and it is estimates of each of these 10 terms, including the coefficients, that are given in Table 6. The variance of each effect when gene frequencies are one half is just the effect squared, i.e., $\sigma^2_a = a^2_a$, $\sigma^2_b = b^2_a$. The square of each mean effect in Table 6 is expected to contain some error variance, however, The correction in each term is $(1/48)$ of the error variance. Correspondingly, $(1/48)$ of the error mean square was subtracted from each term which accounts for some of the estimates of variances being negative, but which provides unbiased estimates.

For the alternative treatment of additive by additive effects:

$$4\sigma^2_{(a\delta)_{ab}} + 4\sigma^2_c = 2\sigma^2_{(aa)_{ab}} + 2\sigma^2_{(a/\delta)_{ab}} + 2\sigma^2_{(a\delta)_{ba}} + \sigma^2_{(\delta\delta)_{ab}}.$$ 

### TABLE 6

Mean squares, mean effects and variances for the diallel analysis

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>S.S.</th>
<th>M.S.</th>
<th>Mean Variance</th>
<th>S.S.</th>
<th>M.S.</th>
<th>Mean Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>3</td>
<td>10.0997</td>
<td>.3666**</td>
<td>...</td>
<td>7.9948</td>
<td>2.6650**</td>
<td>...</td>
</tr>
<tr>
<td>$a^2_a$</td>
<td>1</td>
<td>.75499**</td>
<td>.2804</td>
<td>.1571</td>
<td>5.9950**</td>
<td>.2491</td>
<td>.1240</td>
</tr>
<tr>
<td>$a^2_b$</td>
<td>1</td>
<td>2.5389**</td>
<td>.1626</td>
<td>.0527</td>
<td>1.9993**</td>
<td>.1443</td>
<td>.0416</td>
</tr>
<tr>
<td>$(aa)_{ab}$</td>
<td>1</td>
<td>.0109</td>
<td>.0106</td>
<td>.0001</td>
<td>.0005</td>
<td>.0024</td>
<td>.0001</td>
</tr>
<tr>
<td>Specific</td>
<td>6</td>
<td>.5903</td>
<td>.0984**</td>
<td>...</td>
<td>.5167</td>
<td>.0861**</td>
<td>...</td>
</tr>
<tr>
<td>$\delta^2_a$</td>
<td>1</td>
<td>.0307**</td>
<td>.0253</td>
<td>.0005</td>
<td>.0385**</td>
<td>.0283</td>
<td>.0007</td>
</tr>
<tr>
<td>$\delta^2_b$</td>
<td>1</td>
<td>.0444**</td>
<td>.0303</td>
<td>.0008</td>
<td>.0504**</td>
<td>.0324</td>
<td>.0010</td>
</tr>
<tr>
<td>$(a/\delta)_{ab}$</td>
<td>1</td>
<td>.0000</td>
<td>.0002</td>
<td>.0001</td>
<td>.0063</td>
<td>.0081</td>
<td>.0001</td>
</tr>
<tr>
<td>$(a\delta)_{ba}$</td>
<td>1</td>
<td>.1596**</td>
<td>.0407</td>
<td>.0032</td>
<td>.1880**</td>
<td>.0302</td>
<td>.0017</td>
</tr>
<tr>
<td>$(\delta\delta)_{ab}$</td>
<td>1</td>
<td>.3033**</td>
<td>.0562</td>
<td>.0062</td>
<td>.2784**</td>
<td>.0538</td>
<td>.0057</td>
</tr>
<tr>
<td>Maternal</td>
<td>3</td>
<td>.0380</td>
<td>.0127</td>
<td>...</td>
<td>.0182</td>
<td>.0061</td>
<td>...</td>
</tr>
<tr>
<td>$\theta^2_a$</td>
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<td>.0319</td>
<td>...</td>
<td>.0002</td>
<td>.0027</td>
<td>...</td>
</tr>
<tr>
<td>$\theta^2_b$</td>
<td>1</td>
<td>.0023</td>
<td>.0009</td>
<td>...</td>
<td>.0070</td>
<td>.0171</td>
<td>...</td>
</tr>
<tr>
<td>$(\theta\delta)_{ab}$</td>
<td>1</td>
<td>.0113</td>
<td>.0217</td>
<td>...</td>
<td>.0110</td>
<td>.0214</td>
<td>...</td>
</tr>
<tr>
<td>Reciprocal</td>
<td>3</td>
<td>.0070</td>
<td>.0023</td>
<td>...</td>
<td>.0081</td>
<td>.0027</td>
<td>...</td>
</tr>
<tr>
<td>$(\delta\delta)_{ab}$</td>
<td>1</td>
<td>.0035</td>
<td>.0121</td>
<td>...</td>
<td>.0034</td>
<td>.0119</td>
<td>...</td>
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<tr>
<td>$(\delta\delta)_{ba}$</td>
<td>1</td>
<td>.0033</td>
<td>.0118</td>
<td>...</td>
<td>.0001</td>
<td>.0020</td>
<td>...</td>
</tr>
<tr>
<td>$(\theta\theta)_{aab}$</td>
<td>1</td>
<td>.0002</td>
<td>.0026</td>
<td>...</td>
<td>.0046</td>
<td>.0139</td>
<td>...</td>
</tr>
<tr>
<td>$(\alpha\alpha)_{ab}$</td>
<td>1</td>
<td>.0056</td>
<td>.0054</td>
<td>.0000</td>
<td>.0052</td>
<td>.0052</td>
<td>.0000</td>
</tr>
<tr>
<td>$C^*$</td>
<td>1</td>
<td>.0053</td>
<td>.0052</td>
<td>.0000</td>
<td>.0016</td>
<td>.0028</td>
<td>.0000</td>
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<tr>
<td>Error</td>
<td>30</td>
<td>.1874</td>
<td>.0062</td>
<td>...</td>
<td>.1092</td>
<td>.0036</td>
<td>...</td>
</tr>
</tbody>
</table>
The correction for environmental variance in estimating each of these terms is the same as before.

The estimates of the embryonic gene effects are not only of the same sign, but are very similar in magnitude for the two varieties, indicating little or no interaction with background genotypes.

Since maternal as well as reciprocal effects were indicated to be unimportant, there is no need to resort to parts of the factorial analysis.

DISCUSSION

Various procedures are available for testing for epistatic effects of genes. Only when controlled crosses are made involving the genes in question, as was done by FASOULAS and ALLARD (1962), by HUMPHREY, MATZINGER and MANN (1964), and in this study, can the various gene effects be estimated directly. Even then, maternal effects, particularly if they interact with embryonic effects, may render the analysis uninterpretable. To clearly separate maternal and embryonic effects one needs all offspring genotypes in association with each maternal genotype, an impractical if not impossible requirement in a study of characteristics of seed. Fortunately, in plant species, maternal effects appear to be fairly rare.

The diallel analysis does provide tests of interactions of maternal with embryonic effects, and further of maternal effects. While these tests are not as sensitive as one might wish, they do afford some protection against wrong inferences concerning the embryonic gene effects. Only when the maternal effects do not interact with embryonic gene effects (a shaky conclusion at best) are the maternal and embryonic gene effects separable. Also, it is only then that part of the factorial analysis (male) is resorted to for part of the estimates ($a'$s and $aa'$).

As for the results of this study, it seemed safe to conclude that there were no maternal effects. On the other hand, all embryonic gene effects except additive by additive were found to be significant. As can be seen from the means (Table 6), the additive effects are manyfold larger than most of the others, and the additive effect for $Gl_2 = a_o$ is almost twice as large as that for $Gl_3 = b_o$. Probably the best way to view the relative importance of the various effects is in terms of their corresponding variances. For example, in a noninbred linkage equilibrium population, $Gl_2$ would contribute about three times as much variation as $Gl_3$ (.1571/.0527 for Coker and .1240/.0416 for Empire). That most of the variation is additive can be seen from comparing the various classes:

<table>
<thead>
<tr>
<th></th>
<th>Coker</th>
<th>Empire</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Variance</td>
<td>Total percent</td>
</tr>
<tr>
<td>Additive</td>
<td>.2098</td>
<td>94</td>
</tr>
<tr>
<td>Dominance</td>
<td>.0013</td>
<td>01</td>
</tr>
<tr>
<td>Epistatic</td>
<td>.0104</td>
<td>05</td>
</tr>
<tr>
<td>Totals</td>
<td>.2215</td>
<td>100</td>
</tr>
</tbody>
</table>
While highly significant, dominance and epistasis together contribute only 6% of the total variation.

The alternative treatments of the additive by additive effects have been purely illustrative since the two loci are independent. As a means of testing for a configuration effect one would test the comparison $C^*$. As a means of expressing the genetic variance and the covariances of relatives in noninbred populations there are advantages of simplicity in using $(aa)$ and $(a/a)$ instead of $(aa)$ and $C$. In either case, one must reckon with product terms in the case of some relatives.

**SUMMARY**

The effects of genes at two independent loci, identifiable by their gland-producing pattern, on level of seed gossypol were studied. The experiment consisted of all possible crosses, including selfs, of the four homozygous genotypes, repeated in two Upland varietal backgrounds of *Gossypium hirsutum* L., and grown in three replications. Gossypol level was determined for seed harvested from the mother plant.—Alternative analyses, factorial and diallel, were compared for a genetic model which included direct maternal gene effects, embryonic gene effects and their interactions. The main sources of variation for the diallel analysis were further broken down into individual degree of freedom comparisons, each of which was related to its content of gene effects. It was shown that only when maternal and embryonic gene effects do not interact can the two be separated and estimated unbiasedly by using parts of both the factorial and diallel analyses. When there are no maternal effects, however, the diallel analysis provides all the information about embryonic gene effects. Two treatments of additive by additive effects were considered, which take into account chromosome configuration effects.—Application of the analyses to the data on gossypol level showed no maternal effects, highly significant dominance and epistatic effects but which when combined accounted for only 6% of the genetic variance, and one locus, $Gl_2$, to contribute about three times as much additive variance as the other locus, $Gl_3$.

**LITERATURE CITED**


