

Linkage Relationships between *Le* and *G1* Alleles in Cotton¹

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ABSTRACT

The major leaf-gland alleles of cotton, *Gossypium hirsutum* L., *G1₂* and *G1₃*, were shown to be linked with the complementary lethality alleles, *Le₁* and *Le₂*. Linkage between *G1₂* and *Le₁* was estimated at 27.5 ± 2.8 centimorgans, and linkage between *G1₃* and *Le₂* was estimated at 22.9 ± 2.2 centimorgans. Because the complementary lethality allele from *G. davidsonii* Kell., *Le^{dav}*, is also linked with the *g1₃* locus of *Gossypium*, the question arose as to whether or not *Le^{dav}* and *Le₂* are alleles at a common locus. Direct methods of testing are not possible inasmuch as combining the two alleles in a zygote results in the death of the embryo, or the plant, stemming therefrom. However, indirect results as well as reconstruction of probable pathways in the evolution of alleles at the *le* loci in various *Gossypium* taxa led to the tentative conclusion that *Le₂* and *Le^{dav}*, although antagonistic in combination, are alleles at a common locus.

Additional index words: *Gossypium anomalum* Wawra \times Wawra and Peyr., *G. arboreum* L., *G. barbadense* L., *G. davidsonii* Kell., *G. herbaceum* L., *G. klotzchianum* Anderss., *G. hirsutum* L., *G. sturtianum* J. H. Willis, *G. stocksii* Mast. in Hook., Complementary lethality, Duplicated linkage.

EARLIER, I demonstrated linkage between the *G1₃^{dav}* allele of the wild diploid taxon *Gossypium davidsonii* Kell. and a complementary lethality factor, *Le^{dav}* (8). The work was performed using the background of *G. barbadense* L., one of the two cultivated tetraploid species of cotton; and linkage was estimated at 25.9 ± 3.0 centimorgans. Because *Le^{dav}* interacts with *Le₁* and *Le₂* of the tetraploid cottons to promote either embryonic or seedling lethality (9), I speculated that *Le^{dav}* might be allelic with one of those complementary factors. Because of the lethality complication, direct methods of testing were not possible. However, allelism might be inferred if either *Le₁* or *Le₂* were shown to be linked with the *g1₃* locus of the cultivated cottons and if the linkage estimate were similar to that for *Le^{dav}* and *G1₃^{dav}*.

MATERIALS AND METHODS

Detection of Linkage

NC-150-Compatible is a glandless line of *G. hirsutum* L. of the genotype *g1₂g1₂g1₃g1₃; le₁le₁le₂le₂*. The cotton thus produces viable offspring when crossed with a stock homozygous for *Le^{dav}* (7). 'Coker 310' is a cultivar of *G. hirsutum* L. of the genotype *G1₂G1₂G1₃G1₃; Le₁Le₁Le₂Le₂* (9). This normally glanded cotton produces abortive embryos when challenged with the *Le^{dav}* allele. Coker 310 was crossed with NC-150-Compatible, and the F₁ was backcrossed with the glandless parent. The resulting seed were germinated, and six plants of each of the genotypes *G1₂G1₂g1₃g1₃* and *g1₂g1₂G1₃G1₃* were selected according to cotyledonary gland scoring procedures outlined by Lee (5). The 12 plants were grown in a greenhouse; and each was crossed with NC-150-Incompatible, a glandless stock of *G. hirsutum* homozygous for *Le^{dav}* (7). The seed from all crosses were germinated in a soil flat, and the resulting seedlings were scored for presence of cotyledonary glands and lethality interaction.

Four of the six plants harboring *G1₂* showed an association between *G1₂* and *Le₁*, i.e., most of the gland-bearing seedlings

Table 1. Phenotypic ratios and linkage estimates for two segregating populations of cotton.

Genotype of female parent†	Seedling phenotype	No. in class	Linkage estimate
<i>G1₂g1₂g1₃g1₃; le₁le₁le₂le₂</i>	Glanded viable	88	27.5 ± 2.8
	lethal	29	
	Glandless viable	39	
	lethal	92	
<i>g1₂g1₂G1₃G1₃; le₁le₁Le₂le₂</i>	Glanded viable	140	22.9 ± 2.2
	lethal	34	
	Glandless viable	48	
	lethal	136	

† Genotype of male parent, NC-150-Incompatible, is *g1₂g1₂g1₃g1₃; le₁le₁Le^{dav}Le^{dav}*.

became necrotic and moribund within 5 days after germination (9). Another plant displayed evidence of harboring both *Le₁* and *Le₂*, and the sixth produced seedlings that were all viable.

Five of the six plants harboring *G1₃* showed evidence of an association between *G1₃* and *Le₂*; that is to say, most of the gland-bearing seedlings became necrotic and moribund by day 10 after germination (9). The sixth plant produced seedlings that were all viable.

Estimating Degree of Linkage

The two plants that produced all viable seedlings were saved and crossed with the following stocks: the plant harboring a single allele of *G1₂* was crossed with a glandless stock of the genotype *Le₁Le₁le₂le₂*, and the plant bearing a single allele of *G1₃* was crossed with a glandless stock of the genotype *le₁le₁Le₂Le₂*. Gland-bearing seedlings from the crosses should have, in the first combination, *G1₂* and *Le₁* in repulsion, and in the second, *G1₃* and *Le₂* in repulsion. Three seedlings of each of the genotypes were grown to maturity, and all plants crossed with glandless NC-150-Incompatible. The resulting seed were germinated in soil flats, and the seedlings were scored for glandulosity and lethality interaction.

RESULTS AND DISCUSSION

The results are listed in Table 1. Apparently, the relationships between the *G1* and the *Le* alleles are yet another example of duplicated linkage in *G. hirsutum*. Each of the linkage groups involved, V in the A subgenome and IX in the D, consists of three duplicated factors (4). Such is perhaps not surprising in an allotetraploid species like *G. hirsutum*.

The recombination fractions, 22.9 ± 2.2 centimorgans for *G1₃* and *Le₂* and 25.9 ± 3.0 for *G1₃^{dav}* and *Le^{dav}* (8), leave little doubt that these two *Le* alleles are in the same linkage group. The *G1₃* and *G1₃^{dav}* alleles were previously placed at a common locus (8). Moreover, the similarity of

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the linkage values suggests that Le^{dav} and Le_2 are also at a common locus. However, attempting to make a conclusive case for allelism using the linkage values reported herein requires caution, considering the backgrounds of the various alleles involved. $G1_3^{dav}$ and Le^{dav} derive from *G. davidsonii*; le_1 and le_2 from *G. barbadense*; and Le_1 , Le_2 , and the contrasting pairs at the gland-determining loci from *G. hirsutum*. Insertion of "foreign" germplasm into the chromosomes of a species can alter recombination fractions, sometimes drastically (6,13). Therefore, the recombination estimates given herein, and that from an earlier report (8), could change as the germplasm blocks accompanying the alleles in question are subjected to further manipulation.

Still, a reconstruction of the probable paths of descent of the alleles at the le loci, taken along with the recombinational evidence, lends credence to the belief that Le^{dav} is allelic with Le_2 . Among the species that display either embryonic or seedling lethality when challenged with the Le^{dav} allele are the A genome species (*G. arboreum* L. and *G. herbaceum* L.), all except one of the D genome species that have been tested, the tetraploid AD taxa, and the E genome species, *G. stocksii* Mast. in Hook. (15,16). The presence of the potential for a lethality interaction among these divergent and geographically dispersed species suggests that the Le alleles represent the primeval state of the genes. Because Beasley (2) showed that the tetraploid cottons have A and D subgenomes and because Le_1 is sited in the A genome and Le_2 in the D, an Old World diploid must have contributed Le_1 and a New World diploid Le_2 . The Le alleles of the tetraploids behave as variants of the basic type, the difference being that one dose of Le in a diploid promotes embryonic death at about the same stage of development as Le_1 and Le_2 combined in *G. hirsutum*. The combined action of the two alleles in *G. barbadense* is somewhat tardier than in *G. hirsutum* (9). Alternatively, the action of the Le alleles in the tetraploids could be a matter of dosage compensation. Transfer of an Le from an A or a D diploid to *G. hirsutum* might afford a clue as to whether there is dosage compensation or a state of reduced expression.

As far as has been demonstrated, Le^{dav} is found only in *G. davidsonii* and, presumably, in the closely related *G. klotzschianum* Anderss. These species cross readily and produce robust hybrid offspring, and both taxa promote lethality in crosses with a series of *Gossypium* taxa (15). Perhaps the two are merely races of a single species, the D_3 (or *G. klotzschianum*) pair (3). Nonetheless, the two races or species are widely dispersed geographically, *G. klotzschianum* growing in the Galapagos Islands and *G. davidsonii* about the Gulf of California. Although the crosses between the two D_3 cottons and other taxa that have been reported could not have involved a large number of plants, all reports show no exception to the rule that the D_3 cottons are homozygous for at least one complementary lethality factor (7,8,9,11,15,16). Moreover, the presence of the allele in such widely dispersed taxa suggests that Le^{dav} is not a recent mutation.

I can muster one challenge to the claim that the Le^{dav} allele is a variant of the basic Le allele of the D genome. In

a zygote, the combination Le_2Le^{dav} leads ultimately to the death of the embryo or seedling that develops from it. Thus, direct transformation of Le to Le^{dav} , and subsequent survival of the cellular line stemming therefrom, does not seem possible unless both of the Le alleles in a given cell mutated to Le^{dav} simultaneously.

A more likely circumstance is that there was an intermediate state of the Le gene, one not antagonistic to Le^{dav} . Such alleles are known, the le alleles. A few stocks of *G. barbadense* produce viable hybrids with *G. davidsonii* (10,11). I have described the alleles of one such stock as le_1 and le_2 (9). Webber (16) found a stock of Bourbon cotton (a form of *G. hirsutum*) which, after crossing with *G. klotzschianum*, gave seedlings that died in the cotyledonary stage. In my experience, such a result is indicative of monomerism for an Le allele in *G. hirsutum* (9).

Bahavandoss et al. (1) produced viable offspring from a cross of an accession of *G. arboreum* and *G. davidsonii*, thus verifying that mutation to the le state can occur in diploid cottons as well as in tetraploids. Although there is no evidence that an le allele was ever present in the D_3 cottons, the possibility nonetheless exists.

Evidently antagonism between Le^{dav} and at least one of the Le alleles in the tetraploid cottons is not expressed in all environments. Phillips (12) showed that crosses between *G. klotzschianum* and selected strains of *G. hirsutum* and *G. barbadense* died within 2 weeks after germination when grown at temperatures of 32 C or below, whereas at 40 C the hybrids survived without difficulty. At temperatures between 33 and 37 C, the hybrids survived but suffered some defoliation. The accessions of *G. barbadense* and *G. hirsutum* used in Phillips's experiments were not identified, but the fact that hybrid seedlings survived for several days at 32 C suggests that the plants were monomeric, most likely of the genotype $le_1le_1Le_2Le_2$. Therefore, temperature-conditional lethality provides a model for how the combination of $LeLe^{dav}$ might have survived in tissue lines to produce seed homozygous for Le^{dav} .

The African B diploid, *G. anomalum* Wawra ex Wawra and Peyr., and the Australian C diploid, *G. sturtianum* J. H. Willis, hybridize readily with *G. davidsonii* and produce viable offspring (14,15). Presumably these taxa harbor an le allele at the complementary lethality locus, or else the locus is missing. Could an le allele have been the prototype at the locus? The chief evidence against such a circumstance is that le seems to be exceptional in *Gossypium*, whereas Le is widespread.

None of the alleles at the le loci, and here I am assuming that Le^{dav} is one such allele, have any noticeable effects upon plants harboring them. Thus, the various alleles seem to behave essentially as the "wildtype" in their "native" backgrounds. Moreover, Le^{dav} in the background of tetraploid cottons has no discernible effects upon the vigor of plants with the new combination. Therefore, the various alleles at the le loci are detectable only through the route of lethality challenge.

Perhaps the alleles differ only in the juxtaposition of a few codons, differences that do not seem to impair the functions of the genes, but changes that have a dramatic effect when certain of the genes are combined in zygotes.

Modern techniques of biochemical analysis could possibly answer questions of allelism as well as detect changes that have occurred in the various alleles. Stocks on standardized backgrounds are now available for such work.

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